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PHYTOCHEMICAL INVESTIGATION OF ADENIUM OBESUM

FAMILY APOCYNACEAE

by

Joseph John Hoffmann

A Dissertation Submitted to the Faculty of the

DEPARTMENT OF PHARMACEUTICAL SCIENCES

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY
WITH A MAJOR IN PHARMACEUTICAL CHEMISTRY

In the Graduate College

THE UNIVERSITY OF ARIZONA

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THE UNIVERSITY OF ARIZONA

GRADUATE COLLEGE

I hereby recommend that this dissertation prepared under my direction by Joseph John Hoffmann

entitled PHYTOCHEMICAL INVESTIGATION OF ADENIUM OBESUM FAMILY APOCYNACEAE

be accepted as fulfilling the dissertation requirement of the degree of Doctor of Philosophy

J. J. Cole — 7-25-75

Dissertation Director — Date

After inspection of the final copy of the dissertation, the folloving members of the Final Examination Committee concur in its approval and recommend its acceptance:*

J. J. Cole	7-25-75
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ABSTRACT

Cytotoxicity tests carried out in the 9KB (human epidermoid carcinoma of the mouth) tissue culture system demonstrated that the cytotoxic activity resided in a defatted ethanol extract of the stems, leaves and flower of Adenium obesum family Apocynaceae.

After a series of solvent partitions and extractions, the cytotoxic activity was concentrated in a diethyl ether soluble fraction. Through the use of column and preparative plate thin layer chromatography, the following seven compounds were isolated and identified from the active fraction: dihydroifflaionic acid, 3,3'-di-O-methylquercetin, 3-O-methylkaempferol, somalin, honghéloside A, 16-acetylstrospeside, and honghelin.

Infrared and nuclear magnetic resonance spectroscopy as well as mass spectrometry were instrumental in the identification of all seven compounds. Further evidence was provided by melting point determinations of the parent compounds and their derivatives. A carbon, hydrogen analysis was determined for each compound except honghelin whose diacetate derivative was analyzed instead. The structure of dihydroifflaionic acid was confirmed by the preparation of one known derivative and two new derivatives. Microanalytical ultraviolet spectroscopy was used to substantiate the flavonol structures as well as the preparation of one known derivative each for 3, 3'-di-O-methylquercetin and 3-O-methylkaempferol. The unsaturated lactone ring of the four cardenolide

glycosides was confirmed by ultraviolet spectroscopy. Somalin and the deacetylated derivative of hongheloside A were hydrolyzed into their respective genins and carbohydrate moieties. Two known compounds were prepared from 16-acetylstrospeside and honghelin was converted into one known derivative.

Five of the above-mentioned compounds exhibited the following cytotoxic activity (micrograms per ml): 3-3'-di-O-methylquercetin (3.08), somalin (less than 0.01), hongheloside A and 16-acetylstrospeside (0.035), and honghelin (0.02). The other two compounds were inactive in the 9KB test system.

CHAPTER 1

INTRODUCTION

Due to the necessity for new and more effective antineoplastic agents, several thousand plants have been screened as sources of potential antitumor agents. As a result of this ongoing search, an extract of Adenium obesum, family Apocynaceae demonstrated cytotoxic activity against human epidermoid carcinoma of the nasopharynx: 9KB test system (Abbott et al. 1972, p. 17). In addition to concentrating this activity in the diethyl ether soluble fraction, preliminary in vivo tests indicated that the ether soluble fraction has marginal antitumor activity against mouse lymphocytic leukemia: 3PS test system (Abbott et al. 1972, p. 9). Therefore a phytochemical investigation of the active fraction was undertaken.

Occurrence of the Plant

Although the major source of Adenium obesum (Forskal) Roemer and Schultz is the bushlands of East Africa, the plant has been found to grow in the arrid regions of Saudi Arabia, especially in soil composed of volcanic ash (Lavranos 1966, pp. 19-23). The source of the plant investigated was the coastal province of Kenya in the Kilifi District. The plant was collected on November 20, 1972, by R. W. Spjut and P. D. Ensor (#2604) at an elevation below one thousand feet, 39 degrees 55

minutes east, three degrees 12 minutes south in grassy areas or openings of a bushland and thicket region near Jilore. The area was bordered by an open Cynometra forest, Brachystegia forest and Savanna. Adenium obesum was associated with the following plants: Acacia, Carissa, Combretum, Croton, Euphorbia, Onidia, Grewia, Landolphia, Nectaropetalum, Ochna, Salacia, Sclerochiton, Strychnos, Terminalia and Uvaria (Perdue 1975).

Description of the Plant

Adenium obesum can best be described as a succulent shrub with a swollen oddly twisted trunk with thick branches. During the warm moist months the branches have glossy leaves and bright red, five cm bell shaped flowers with a white center. However, during the dry winter months the shrub is nearly leafless and without blossoms (Dodson 1959, p. 55). After further investigation of the botanical description of this unusual plant, a range of forms have been discovered (Lavranos 1966, p. 19-23). The trunk can attain heights of 15 feet and a diameter of 18 inches. The leaves are clustered at the end of branches and have been described as obovate, oblong-obovate or elliptical cuneate. At the base they become sessile or very shortly petioled. In drier areas the leaves are densely tomentose while more inland they become longer, larger and pubescent and sometimes even glabrous. The flowers range up to 60 mm, the corollae up to 25 mm with a cylindrical base of up to 15 mm having a glabrous, pubescent or strigose nature.

Specimens of Adenium obesum are on deposit in the herbarium of the National Arboretum.

Extraction of the Plant

After the stems, leaves and flowers were ground to a coarse powder with a Wiley Mill, 15 kg of the powder was defatted with petroleum ether in a four liter capacity Lloyd type extractor. The defatted marc was subsequently extracted with 95 percent ethanol to yield 1.2 kg of air dried extract which was stored at -10°C.

CHAPTER 2

ISOLATION OF PLANT CONSTITUENTS

An extensive literature search revealed that no previous chemical work had been done on this plant. However, during the search for active cardiac agents, various glycosides of cardenolides were isolated from other Adenium species. Honghe1oside A and C were isolated and characterized by Hunger and Reichstein (1950a) and subsequently by Schindler and Reichstein (1951). Digitalinum verum was isolated as its hexacetate derivative by the previous authors and by Hess, Hunger and Reichstein (1952). However, the actual character of the glycoside in its natural state was never determined. Hunger and Reichstein (1950b) had the same problem with strospeside which was isolated along with 16-desacetyl-anhydrohonghe1oside A and 16-anhydro-strospeside. Somalin was first isolated and characterized by Hartmann and Schlittler (1940). Hess et al. (1952) isolated and identified somalin along with echujin as did Striebel, Tamm and Reichstein (1955) who also isolated and identified honghe1oside A. The appendix lists the structures of the above compounds.

Solvent Partition and Extraction

The ethanol extract of the petroleum ether defatted marc of Adenium obesum was subjected to solvent partitions and solvent extractions (Figure 1).

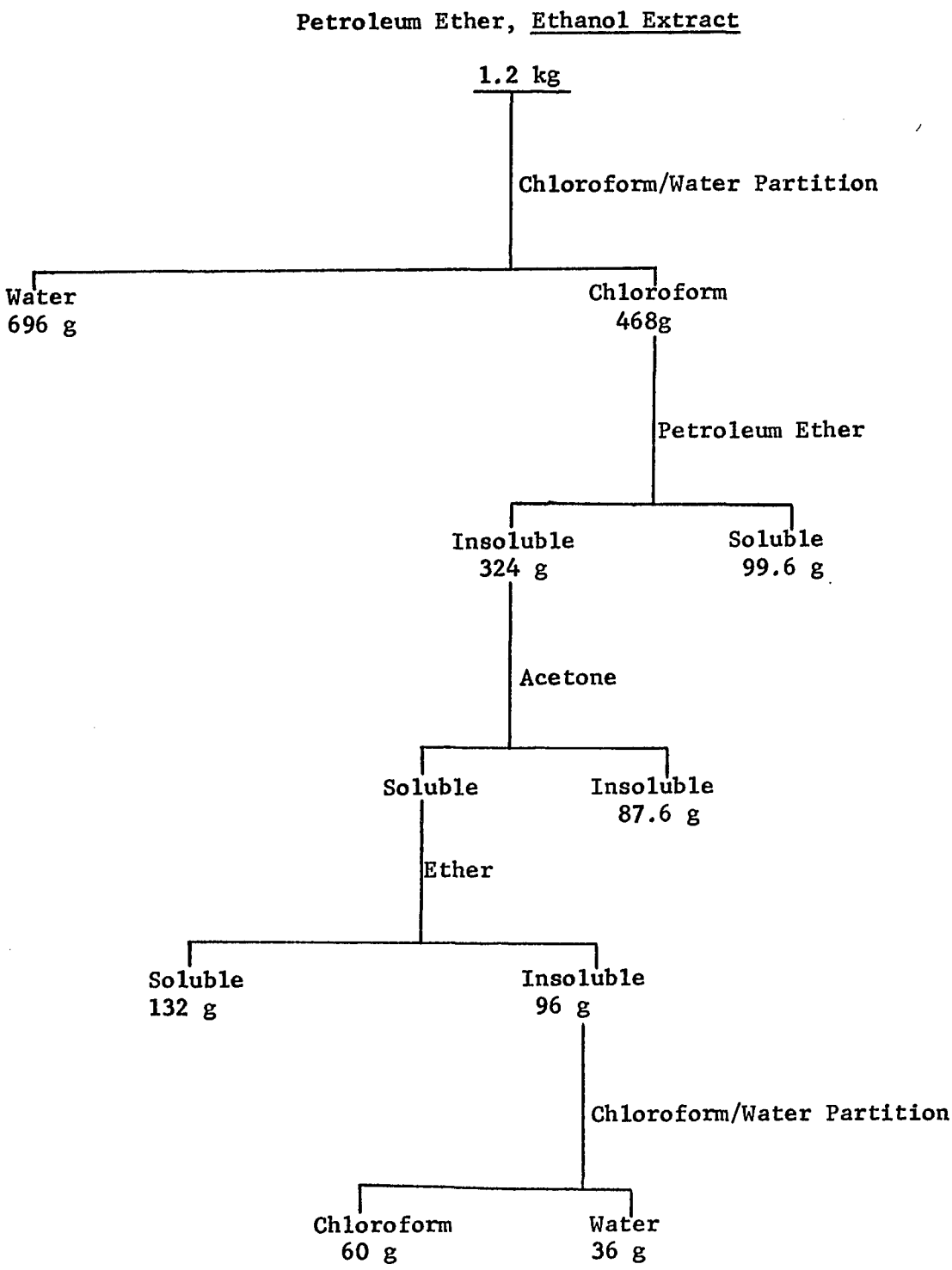


Figure 1. Solvent Partition and Extraction of Adenium obesum.

The preceding procedure resulted in concentrating the KB and PS activity within the ether soluble fraction.

Silica Gel G Column Chromatography

Although chlorophyll was abundant in the ether soluble fraction, an aluminum oxide column was ruled out due to possible degradation of the sensitive glycosidic substances (Fieser and Fieser 1959, p. 761; Hunger and Reichstein 1950a, p. 99). Therefore, the ether extract was further fractionated by eluting a silica gel G column with the solvent system petroleum ether:ether:chloroform (1:3:1). Four distinct fractions (A through D) were separated by combining the tubes collected based on their thin-layer chromatogram pictures (see pages 53-54). Fraction A consisted of a light red spot (compound I) surrounded by brown spots. The major components of fraction B (compounds II and III) were dull yellow under ultraviolet light and bright yellow under visible light after heating. The major component of fraction C (compound IV) was wheat brown under ultraviolet light after 30 seconds of heating and turned dark blue after 90 seconds of heating. The major component of fraction D (compound V) was fluorescent blue-gray under ultraviolet light after 30 seconds, turned yellow under visible light after one minute and turned rust-red after two minutes of heating. However, when the chromatogram was allowed to cool after 90 seconds, a green color was observed. When the chromatograms demonstrated no signs of compound V, the column was eluted clean with acetone.

Isolation of Dihydroifflaionic Acid

Upon treatment of fraction A (a viscous green liquid) with petroleum ether, a dark green powder was recovered by filtration. The green powder was partially dissolved in hot chloroform. The chloroform solution was diluted with an equal volume of petroleum ether and refrigerated overnight. After filtering the cold solution and washing the residue several times with petroleum ether, a light green powder remained. Compound I (dihydroifflaionic acid) was purified by crystallization from methanol. After three recrystallizations from methanol stout white crystals of compound I were obtained.

Isolation of Flavonols

The flavonol mixture was precipitated from fraction B (a viscous green liquid) by the addition of an equal volume of petroleum ether to a chloroform solution of fraction B. The flavonols were separated by preparative thin-layer chromatography with dichloromethane:methanol (96:4) as the developing solvent system. Each of the flavonols was further purified by preparative plate thin layer chromatography with the developing system being chloroform:methanol:water (188:12:1). Flavonol I (compound II) was crystallized from 95 percent ethanol and flavonol II (compound III) was crystallized from absolute methanol.

Isolation of Somalin

Compound IV (somalin) was crystallized directly from fraction C (a green solid) using 20 percent aqueous methanol. Recrystallization from absolute methanol yielded fine white needles.

Isolation of Honghelosíde A

Fraction D was subjected to silica gel G column chromatography with dichloromethane:methanol (96:4) serving as the eluent. Fractions preceding compound V (honghelosíde A) were combined with the mother liquor from fraction C which was subsequently combined with the mother liquor from fraction B. The fractions that contained compound V were further purified by preparative plate thin-layer chromatography with benzene:methanol (9:1) being the developing solvent system. Compound V was crystallized and then recrystallized from methanol and water. The column was then eluted clean with acetone and the material recovered combined with fraction E.

Silica Gel 60 Column Chromatography

The acetone wash from the first silica gel G column was further fractionated by a silica gel 60 column being eluted with the solvent system chloroform:methanol:water (188:12:1). The first several tubes collected yielded an insufficient quantity of a complex mixture based on thin-layer chromatography. The next several tubes were comprised mainly of compound VI based on their chromatograms which exhibited the same color changes as compound V. The next distinct fraction collected was comprised mainly of compound VII based on thin-layer chromatography. Compound VII exhibited the same color changes as compound IV. The later fractions collected overlapped with the ether insoluble fraction.

Isolation of 16-Acetylstrospeside

Fraction four from the silica gel 60 column consisted of mainly compound VI (16-acetylstrospeside). Compound VI was purified by preparative plate thin-layer chromatography with the developing system being ether:chloroform (85:15). Compound VI was then crystallized from aqueous methanol to yield colorless stout plates.

Isolation of Honghelin

Compound VII (honghelin) was concentrated mainly in fraction six from the silica gel 60 column. Purification of compound VII was a result of preparative plate thin-layer chromatography with the developing solvent system being ether:chloroform (9:1). Crystallization from aqueous methanol resulted in the formation of colorless hydrate crystals.

CHAPTER 3

CHARACTERIZATION OF CONSTITUENTS

The seven compounds isolated from the ether soluble fraction were identified by a combination of physical methods, preparation of derivatives and comparison with previously reported data and/or data from authentic samples. This group of seven compounds is comprised of the triterpene hydroxy acid, dihydroifflaionic acid (compound I); the two flavonols, 3,3'-di-O-methylquercetin (compound II) and 3-O-methylkaempferol (compound III); and the following cardenolide glycosides: somalin (compound IV), honghеloside A(compound V), 16-acetylstrospeside (compound VI), and honghelin (compound VII).

Dihydroifflaionic Acid

The crystals of compound I obtained after three recrystallizations from methanol had a melting point range of 259-260°C. This was in very close agreement with the reported melting point of 260°C (Newman 1972, p. 265).

Infrared Spectrum

The infrared spectrum of compound I (Figure 2) was indicative of a hydroxy acid (Nakanishi 1966, pp. 30, 43; Silverstein and Bassler 1967, pp. 89-91) with hydroxyl stretching 3400-3500 cm^{-1}, carbonyl

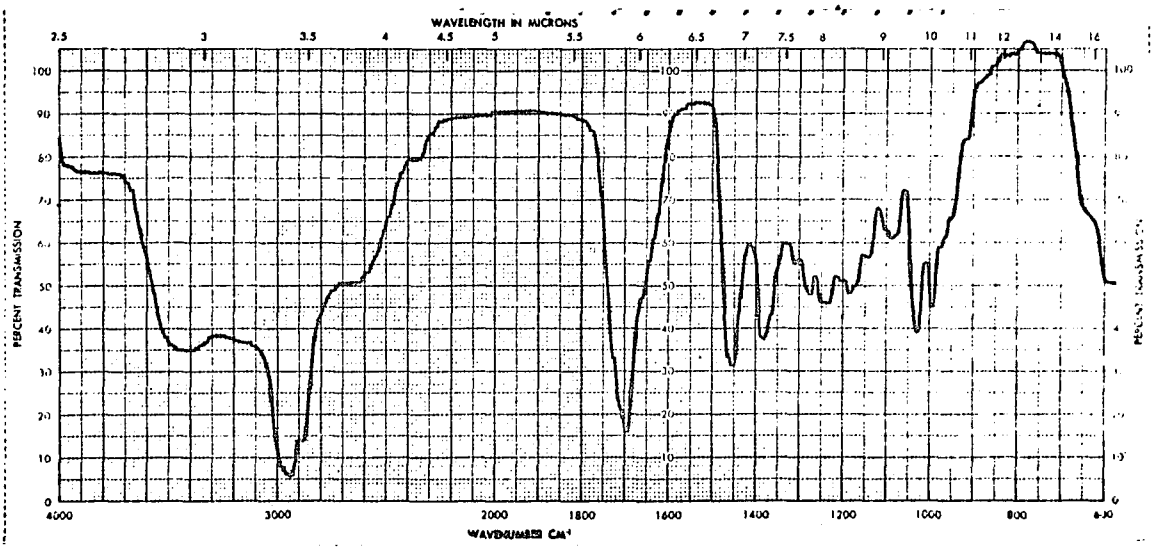


Figure 2. Infrared Spectrum of Dihydroifflaionic Acid.

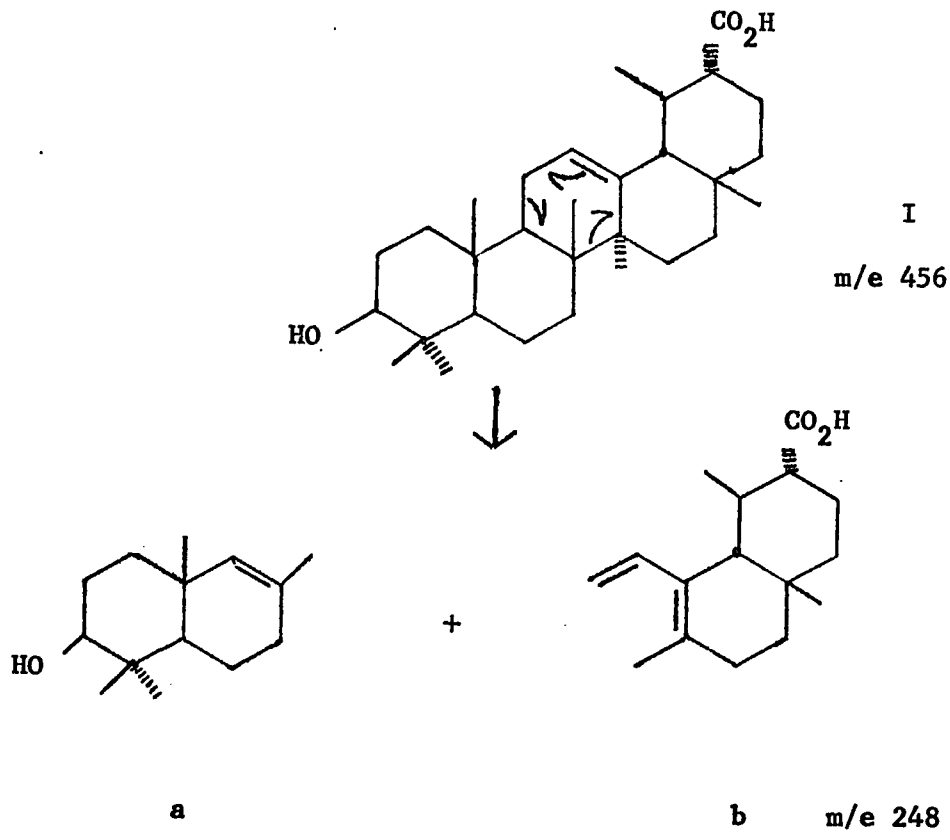
stretching near 1700 cm^{-1}, acid C-O stretching as a doublet centered at 1280 cm^{-1} and acid O-H bending at 1390 cm^{-1}. Furthermore, the hydroxyl group at the three position is in the equatorial conformation since the C-O stretching exhibits absorption at 990 and 1020 cm^{-1} coupled with shouldering from 1030-1040 cm^{-1} compared with 990 and 1066 cm^{-1} for the axial conformation (Allsop et al. 1956, pp. 4869-4872).

Carbon, Hydrogen Analysis

The $C_{30}H_{48}O_3$ molecular formula was substantiated by carbon, hydrogen analysis (calculated: C, 78.89, H, 10.59; observed: C, 78.65, H, 1058) and the mass spectrum parent peak m/e 456.

Mass Spectrum

The mass spectrum of compound I (Figure 3) was indicative of the Δ^{12}-ursenes due to the retro-Diels-Alder reaction producing fragments a and b (Budzikiewicz, Djerassi and Williams 1964, pp. 122-127).



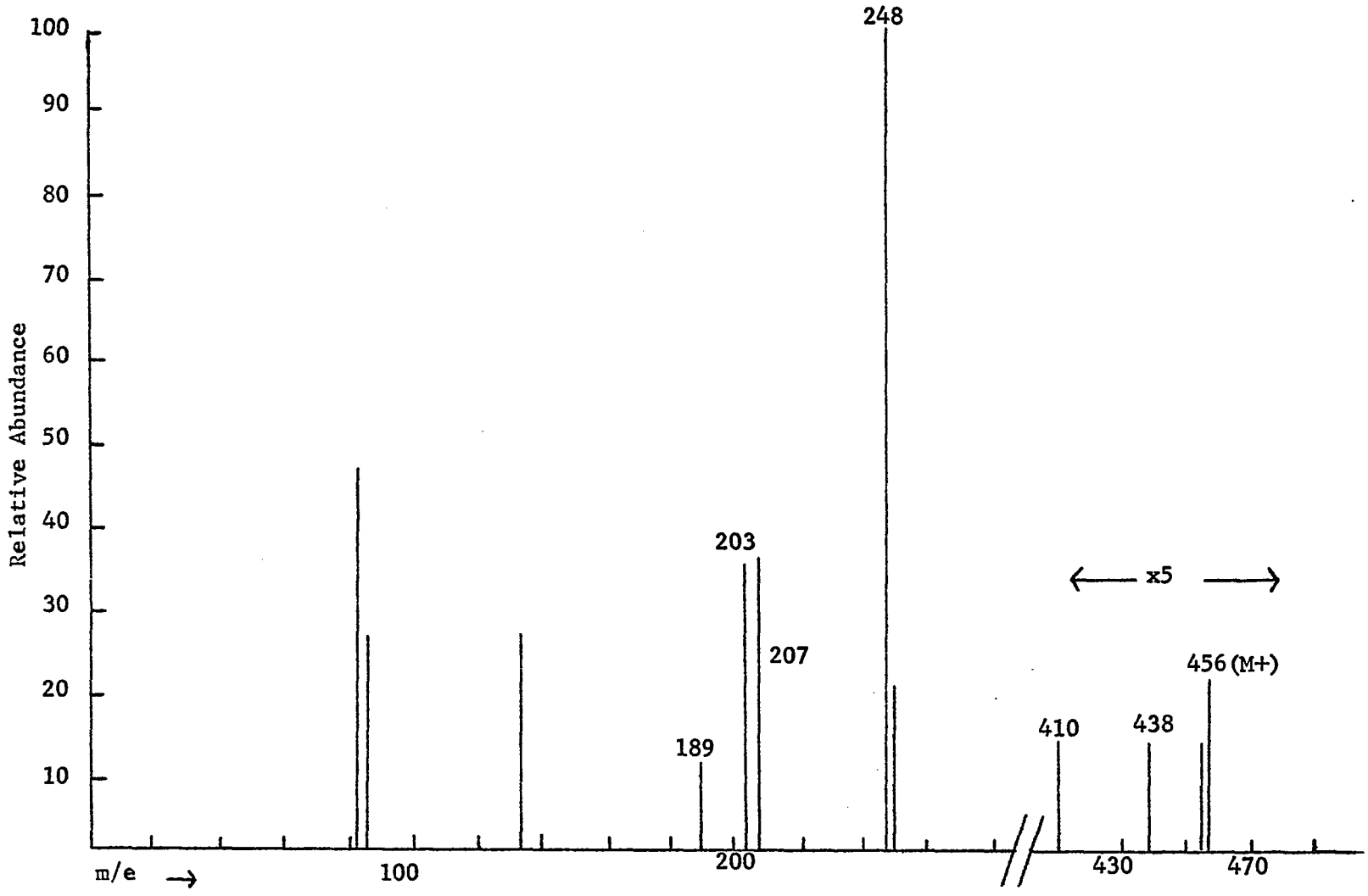
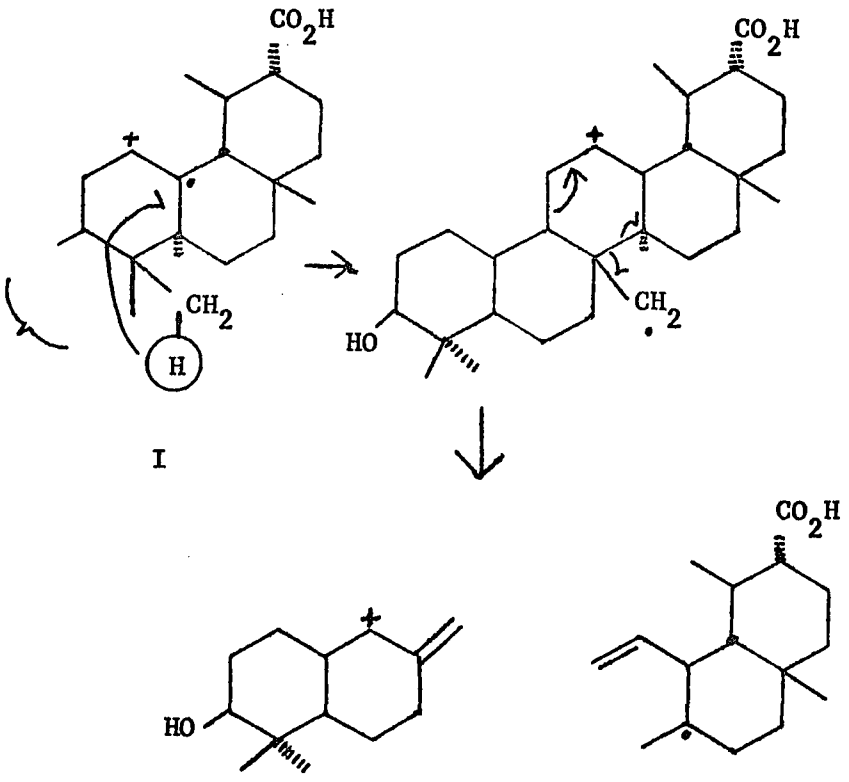


Figure 3. Mass Spectrum of Dihydroifflaionic Acid.

Substituents in fragment a usually do not affect the intensity of the retro-Diels-Alder reaction, while substituents in the rings of b result in the appropriate mass shift of fragment b (Budzikiewicz et al. 1964, p. 125). Thus, b is usually observed as the base peak with major fragments resulting from loss of substituents from b (e.g., loss of C-30 acid group produces peak at m/e 203). However, mass spectrometry studies of ifflaionic acid have shown that the introduction of an electron attracting group at position 20 results in an increased intensity of fragment a less one mass unit (c) due to a proposed competing mechanism of ring C rupture (Shannon 1963, pp. 687-688).



I

c m/e 207

These two mechanisms are consistent with the results obtained from compound I.

Nuclear Magnetic Resonance Spectrum

The nuclear magnetic resonance spectrum for compound I (Figure 4) integrates for one olefinic proton centered at 4.5 delta and one proton at 3.42 delta, a broadened triplet (16 cps) due to the three axial proton (Bhacca and Williams 1964, pp. 77-85). The seven methyl groups can be accounted for as follows: the gem dimethyl group, singlets at 1.23 delta; C-27 methyl, singlet at 1.01 delta, a methyl group alpha to a double bond (Silverstein and Bassler 1967, p. 136); singlet at 0.80 delta, angular methyl at C-6; a singlet at 1.10 delta due to angular methyls at C-8 and C-17 overlapped with doublet at 1.00 delta (J = 5 cps) due to C-19 methyl.

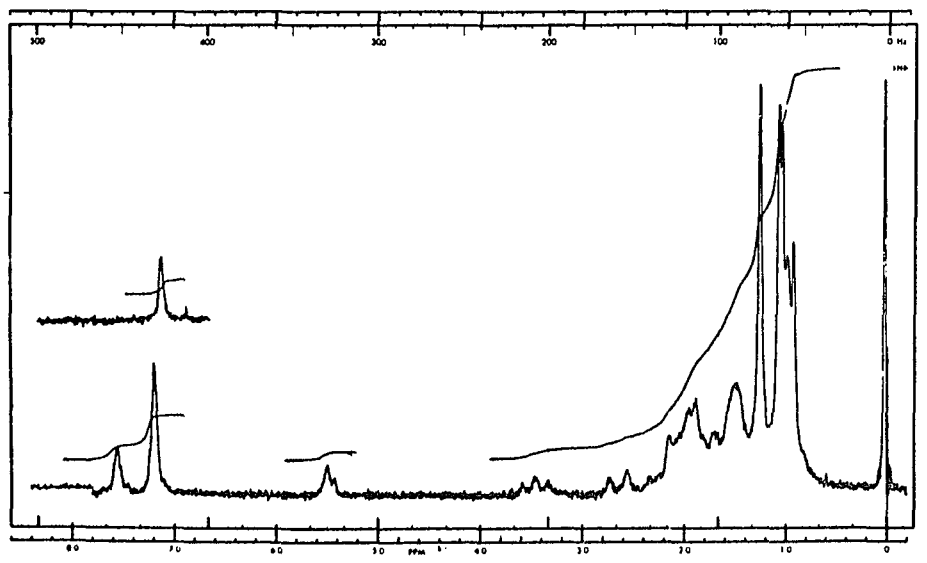


Figure 4. NMR Spectrum of Dihydroifflaionic Acid.

Methyl Ester Derivative of
Dihydroifflaionic Acid

After recrystallization from methanol, the methyl ester derivative of compound I had a melting point range of 209-210°C comparable to the reported melting point range of 207-209°C (Bosson et al. 1963, p. 495). The mass spectrum of the methyl ester (Figure 5) exhibited a parent peak m/e 470 and a peak at m/e 262 (90 percent) consistent with the addition of 14 mass units to the carboxyl group. The formation of the methyl ester was further verified by the shift of the carbonyl absorption to 1730 cm^{-1} and the appearance of an absorption band at 1130-1140 cm^{-1} in the infrared spectrum (Nakanishi 1966, p. 44).

Acetate Derivative of
Dihydroifflaionic Acid

The acetate derivative of compound I had a melting point range of 189-191°C. The mass spectrum of the acetate (Figure 6) had a very weak parent peak m/e 498 but the disappearance of fragment c (m/e 207) coupled with intensification of c-60 peak m/e 189 was consistent with the addition of an acetate group. The infrared spectrum confirmed the acetate primarily by the appearance of an absorption maximum at 1245 cm^{-1} (Nakanishi 1966, p. 44).

Methyl Ester of Acetyldihydroifflaionic Acid

The methyl ester of the acetate derivative of compound I (mp. 228-230°C) was prepared in order to obtain a nuclear magnetic resonance spectrum (Figure 7). The expected peaks at 3.60 and 2.03 delta were consistent for methyl ester and acetate respectively. Furthermore, the

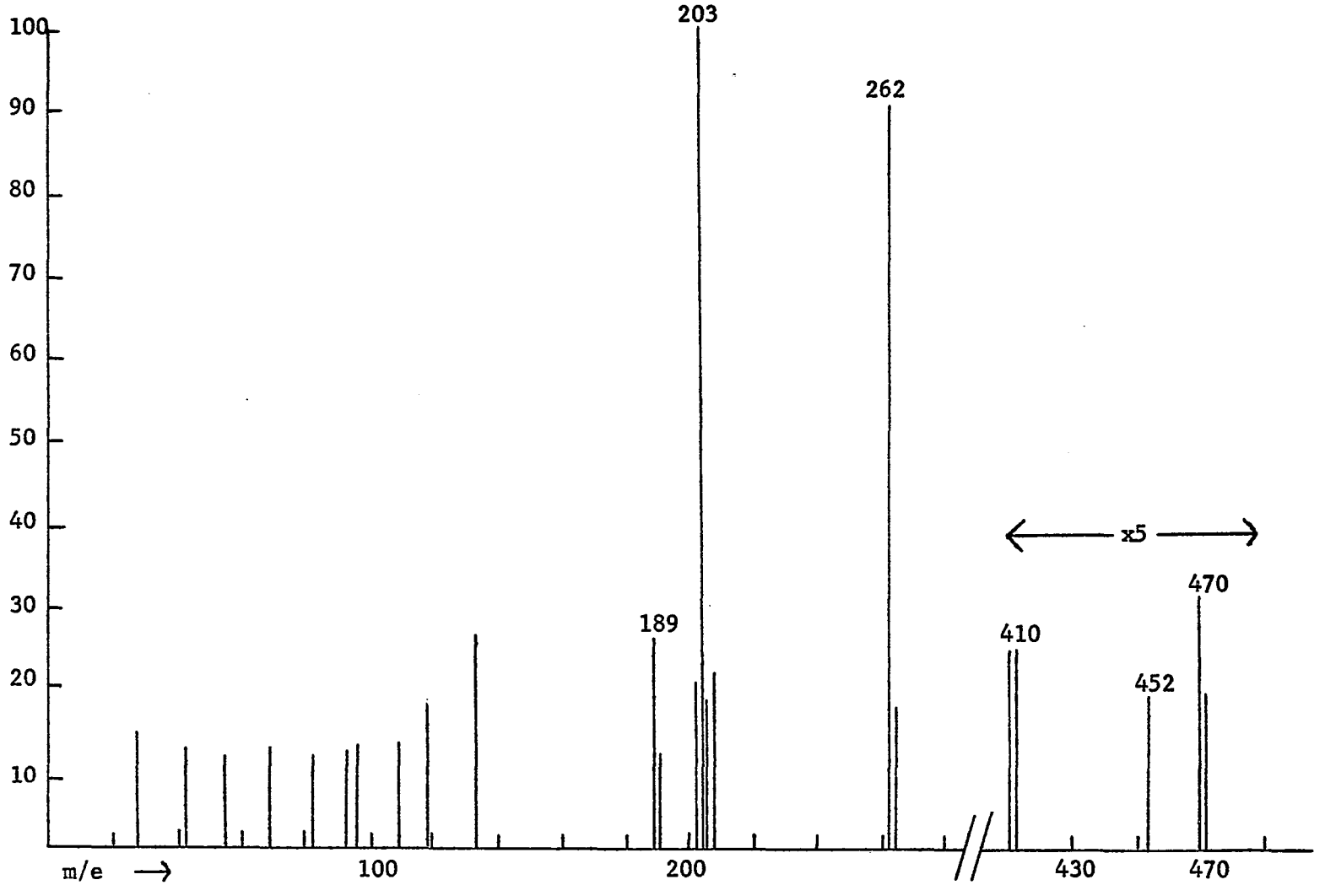


Figure 5. Mass Spectrum of Dihydroifflaionic Acid Methyl Ester.

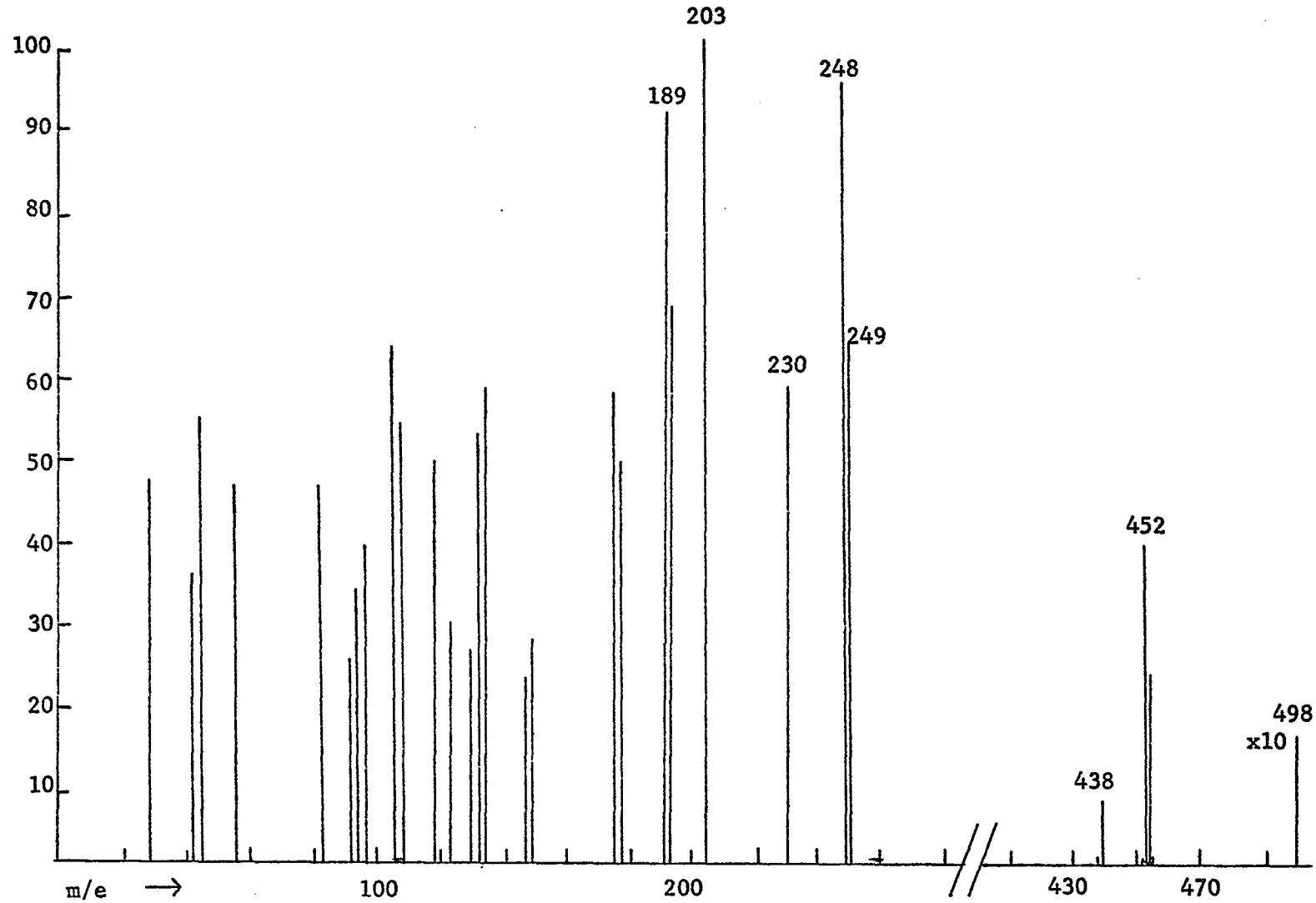


Figure 6. Mass Spectrum of Acetyldihydroifflaionic Acid.

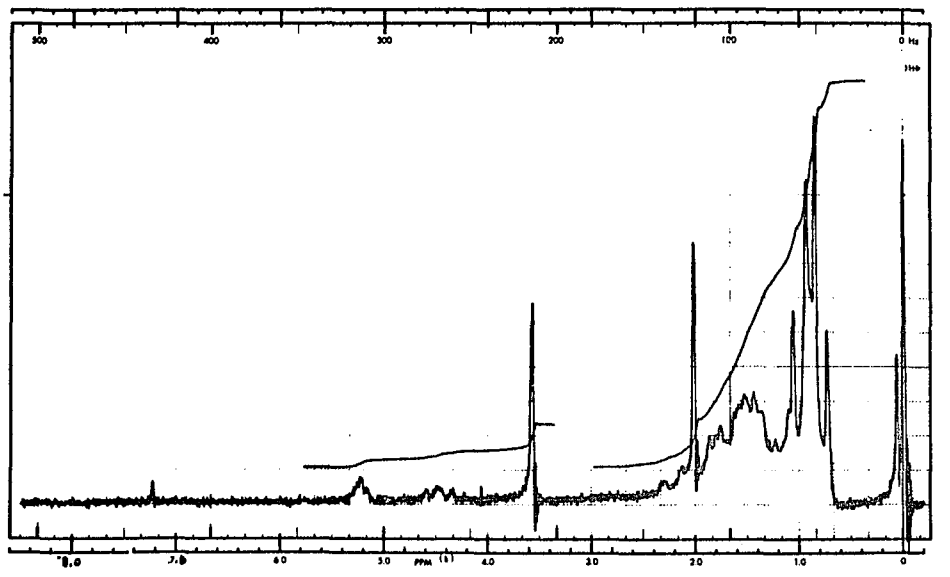


Figure 7. NMR Spectrum of the Methyl Ester of Acetyldihydroifflaionic Acid.

expected chemical shift of the 3-axial proton to 4.50 delta (16 cps) was observed (Bhacca and Williams 1964, p. 77).

3,3'-Di-O-Methylquercetin

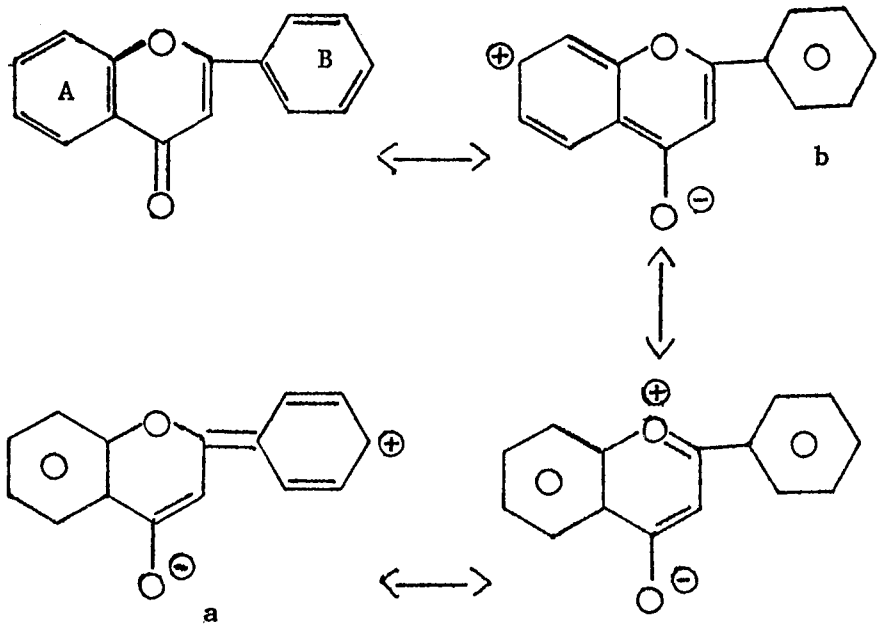
The crystals of 3,3'-di-O-methylquercetin (compound II) obtained were yellow needles with a melting point range of 256-259°C (reported: 258-259°C, Chem. Abs., 1963, p. 1426e).

Carbon, Hydrogen Analysis

The $C_{17}H_{14}O_7$ molecular formula was substantiated by the carbon, hydrogen analysis (calculated: C, 61.82, H, 4.28; observed: C, 62.14, H, 4.31) and the mass spectrum parent peak m/e 330, which was also the base peak. The mass spectra of flavonols exhibit little fragmentation due to their stability (Pelter, Stainton and Barber 1965, pp. 262, 268). Therefore, the parent peak is the base peak and the other major peak is due to the loss of CO, m/e 302, 12% (Reed and Wilson 1963, pp. 5954-5955). However, compound II has methoxy groups present, and therefore an additional stronger peak (m/e 315, 36%) was observed due to loss of a methyl group.

Ultraviolet Spectrum

A multitude of structural information can be obtained from the ultraviolet spectra of flavonols by observing characteristic shifts of band I (320-380 nm) and band II (240-270 nm) of flavonols due to the effects of various reagents on the substitution patterns of the parent flavonol (Mabry, Markham and Thomas 1970, pp. 35-61). This phenomenon can be explained as being due to the resonance contribution of a and b to the ultraviolet spectrum:



Band I is associated with a and undergoes a bathochromic shift with the introduction of hydroxyl groups in ring B. Band II is associated with b and has a similar effect as band I when hydroxyl groups are substituted in ring A (Geissman 1962, pp. 107-127).

The ultraviolet spectrum of compound II λmax (log ε): 359 (4.28), 266 (4.20), and 256 nm (4.30), was almost identical to the previously reported spectrum, λmax (log ε): 360 (4.31), 268 (4.24), and 256 (4.31) (Geissman 1962, p. 112). The fact that band II occurs as a double λmax at 256 and 266 nm is consistent with the observed disubstitution pattern in ring A of 4'hydroxy group and 3'methoxy group. Upon the

addition of sodium methoxide, the expected shifts of a polyhydroxy flavonol were observed: λmax (log ε), 410 (4.38) 322 (3.96) and 274 nm (4.33). Alcoholic sodium acetate is much more selective and will only ionize hydroxy groups at positions 7, 3 and 4'. Band I was shifted to 383 nm and band II was shifted to 277 nm, due to ionization of seven hydroxyl group, e.g., kaempferol's band II was shifted to 275 nm (Geissman 1962, p. 123).

The location of the methoxy groups was consistent with the fact that after one hour the spectrum of compound II in a methanolic sodium methoxide solution was unchanged and that a spectrum run in a methanolic aluminum trichloride solution was stable. Therefore, one of the methoxy groups must be at position 3 (Mabry et al. 1970, pp. 47, 51-55; Geissman 1962, p. 126). Furthermore, since upon the addition of hydrochloric acid to the latter solution no change in the spectrum (λmax: 405, 365, 277 and 268 nm) was observed, the other methoxy group must be at position 3'. An additional verification of the 3' methoxy group was the fact that the spectrum of compound II in a boric acid plus sodium acetate solution was superimposable with the original spectrum of compound II in a methanol solution (Jurd, 1956, p. 376).

Nuclear Magnetic Resonance Spectrum

The nuclear magnetic resonance spectrum of compound II (Figure 8) is very representative of the quercetin type flavonols (Mabry et al. 1970, pp. 294-301). The spectrum integrates for two methoxy groups at 3.80 and 3.87 delta, one hydrogen bonded phenolic group at 12.73

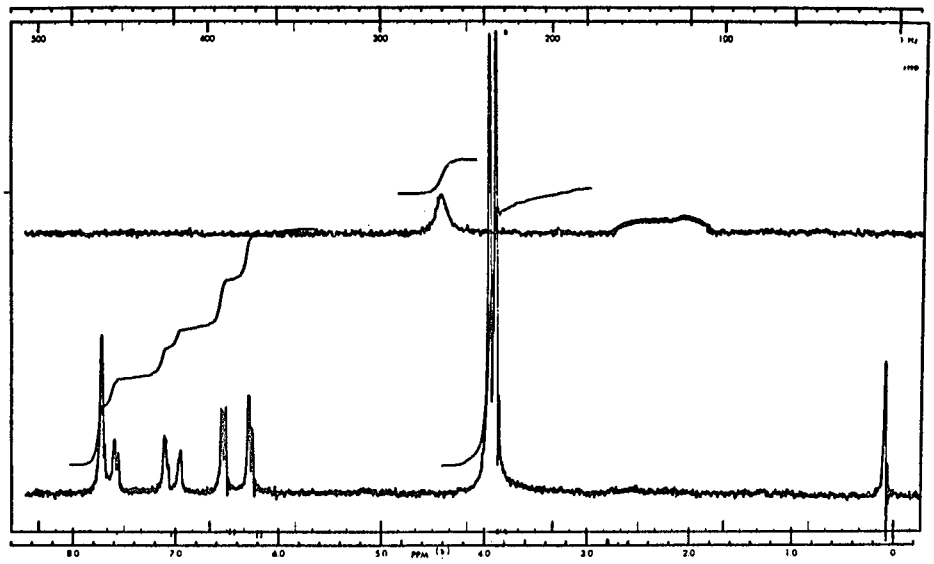
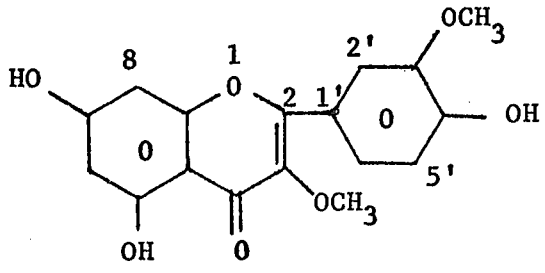


Figure 8. NMR Spectrum of 3,3'-Di-O-methylquercetin.



II

delta (5-OH) and two phenolic protons exhibiting broad bands centered at 10.80 and 10.40 delta. The coupling patterns of the aromatic protons (Silverstein and Bassler 1967, p. 145) are indicative of structure II: two protons with meta splitting pattern (J = 2 cps), H-6 at 6.20 and H-8 at 6.45 delta; one proton centered at 6.97 delta (doublet of doublets, J = 7 cps, ortho coupling; J = 1 cps, para coupling); two protons centered at 7.57 delta whose pattern breaks down to doublet at 7.50 delta (J = 2 cps) due to 0.5 protons and a semi-doublet pattern at 7.62 delta (J = 1 cps) due to 1.5 protons, therefore, 7.62 delta pattern is due to H-2' plus part of H-6' which is now centered at 7.56 delta (J = 7 cps).

Infrared Spectrum

The infrared spectra of flavanones and flavones have been given extensive study in regard to carbonyl, hydroxyl stretching and C-H out-of-plane bending absorption (Briggs and Colebrook 1962, pp. 939-957). The spectrum of compound II (Figure 9) is consistent with these studies. The carbonyl absorption appears at 1650 cm^{-1} as previously reported (Briggs and Colebrook,1962,p. 946). The hydroxyl absorption tends to vary considerably and in this case can be seen as a broad band 3100-3190 cm^{-1}. The C-H out-of-plane bending fluctuates somewhat but the reported absorption at 880 cm^{-1} which is weaker than the absorption at 810 cm^{-1} (Briggs and Colebrook,1962,p. 954) is consistent with the spectrum of compound II.

Triacetate Derivative of 3,3'-Di-O-methylquercetin

The triacetate derivative formed fine white needles when crystallized from ethanol. The melting point (195°C) was identical to the

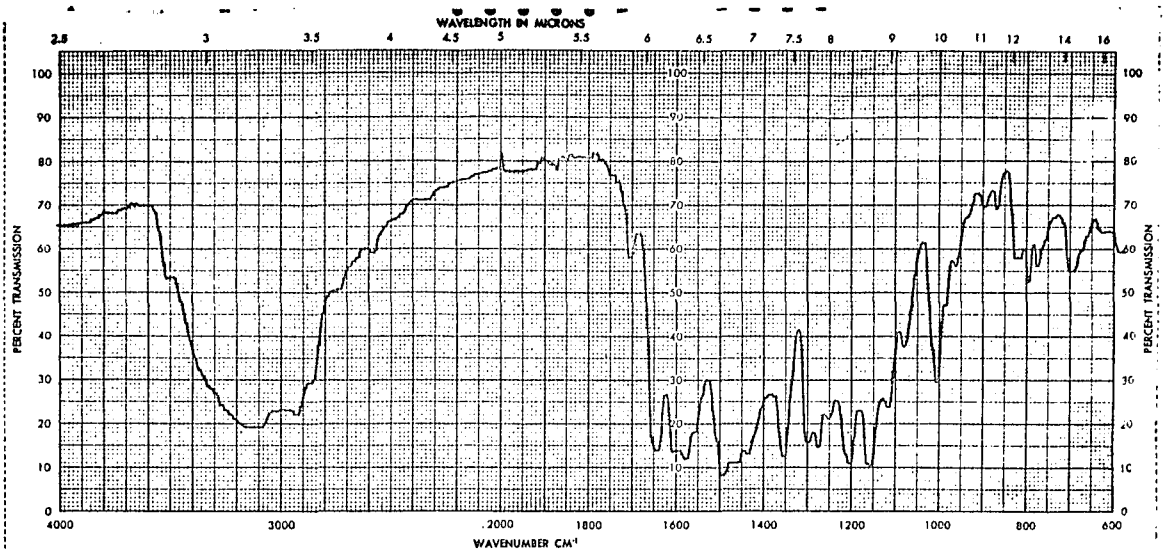


Figure 9. Infrared Spectrum of 3,3'-Di-O-methylquercetin.

previously reported melting point (Chem. Abs. 1962, p. 137113e). The infrared spectrum of the triacetate derivative (Figure 10) indicated the expected disappearance of hydroxyl absorptions, an additional carbonyl absorption at 1760 cm^{-1} along with an intense absorption at 1200 cm^{-1} (Briggs and Colebrook 1962, p. 944).

3-O-Methylkaempferol

The yellow crystalline platelets of 3-O-methylkaempferol (compound III) obtained from absolute methanol had an identical melting point range (270-272°C) as previously reported (Visweswara and Seshadri

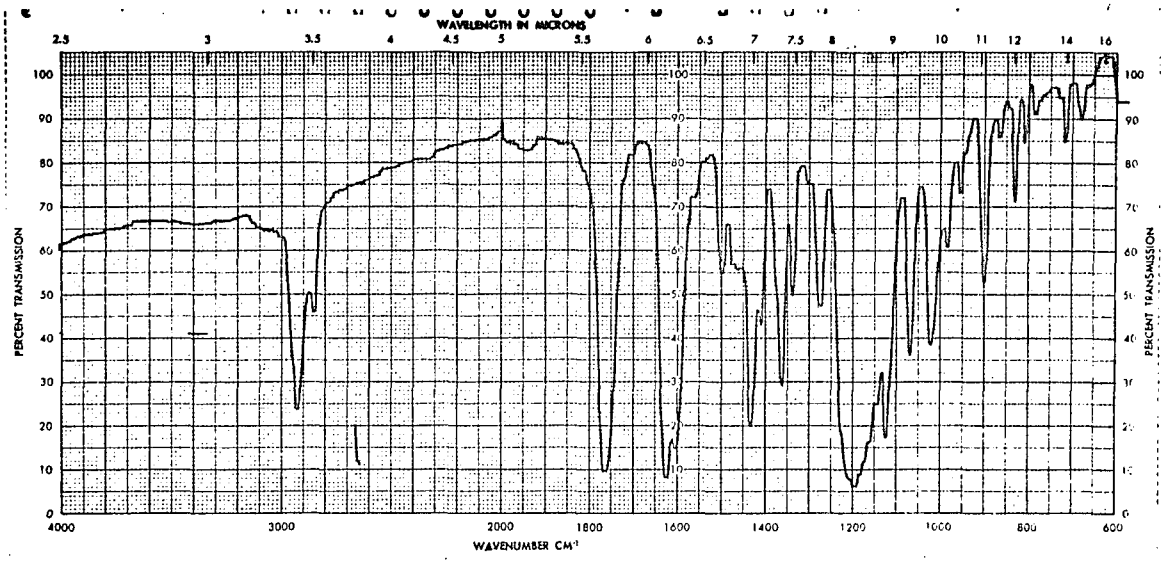


Figure 10. Infrared Spectrum of 3,3'-Di-O-methylquercetin Triacetate.

1947, p. 124). Since compound III differs from the previous compound by the absence of the 3'methoxy group, the approach to its structural identification was identical.

Carbon, Hydrogen Analysis

The molecular formula $C_{16}H_{12}O_6$ was substantiated by the carbon, hydrogen analysis (calculated: C, 64.00, H, 4.03; observed: C, 64.21, H, 4.16) and mass spectrum parent peak m/e 300. As previously noted, the parent peak was also the base peak. The unexplained strong M-1 peak (m/e 299, 90%) as previously reported (Budzikiewicz et al. 1964, p. 262)

and the m/e 257 (54%), due to loss of CO and methyl group, were the only other strong peaks in the mass spectrum of compound III.

Ultraviolet Spectrum

The ultraviolet spectrum data obtained for compound III was in very close agreement with previously reported data (Pacheco and Grouiller 1965, p. 781). The observed spectra were of compound III in a methanol solution while the reported spectra were of 3-O-methylkaempferol in an ethanol solution, λmax (log ϵ), reported: 268 (4.29), 299 (4.08) and 353 nm (4.29); observed: 270 (4.26), 300 (4.06) and 350 nm (4.24); after addition of sodium methoxide, reported: 274 (4.36), 325 (4.12) and 398 nm (4.42); observed: 276 (4.33), 325 (4.05) and 398 nm (4.35); after addition of aluminum trichloride, reported: 277,304,345, and 397 nm; observed: 276,305,352, and 400 nm.

By relying on the information already collected during the identification of compound II, the spectral results of compound III are readily apparent. Since band II occurs as a single absorption maximum, the 3'methoxyl group must be absent. The spectra of compound III demonstrated no decomposition after one hour in sodium methoxide solution and aluminum trichloride solution. Therefore, the single methoxy group must be present at the three position. Furthermore, when the spectrum was of its sodium acetate solution, band II exhibited the expected bathochromic shift to 276 nm.

Nuclear Magnetic Resonance Spectrum

The nuclear magnetic resonance spectrum of compound III (Figure 11) was indicative of its proposed structure. The positions of the three phenol groups were the same as in compound II.

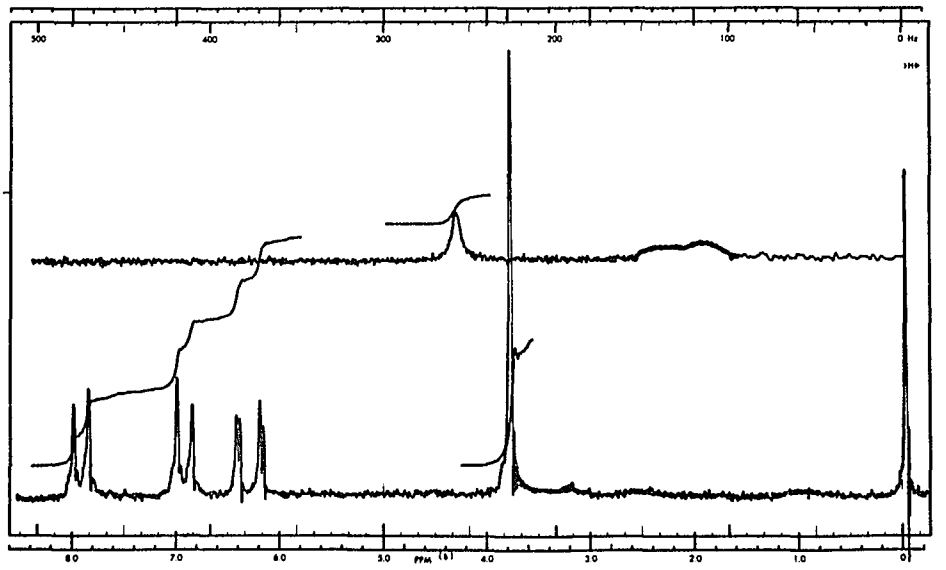
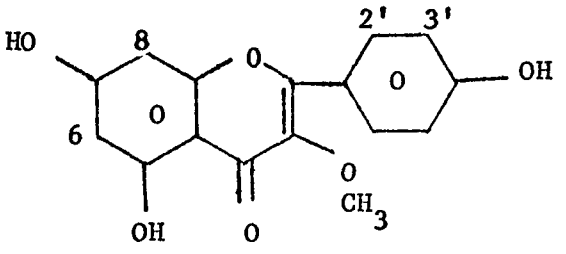


Figure 11. NMR Spectrum of 3-O-Methylkaempferol.



III

The single methoxy group appeared at 3.80 delta. The aromatic protons H-6 (6.20 delta) and H-8 (6.45 delta) were superimposable with the previous spectrum. The remaining aromatic protons' pattern changed to an apparent doublet of doublets due to the ortho, meta and para splitting by each proton and the symmetrical nature of H-2' and H-6' as well as H-3' and H-5'. The doublet centered at 7.95 delta is due to H-2' and H-6' while the doublet centered at 6.88 delta is due to H-3' and H-5'.

Infrared Spectrum

The infrared spectrum of compound III (Figure 12) is typical of the polyhydroxy flavonols. The carbonyl absorption is at 1650 cm^{-1} and the hydroxyl absorption is concentrated at 3300 cm^{-1}. The reason for the better resolution of the hydroxyl absorption is probably its dependence on concentration (Brand and Eglington 1965, p. 136).

Triacetate Derivative of 3-O-Methylkaempferol

The triacetate derivative formed yellow needle-like crystals in petroleum ether and ethyl acetate. The reported melting point range of 161-163°C (Visweswara and Seshadri 1947, p. 124) was reduced to a sharp melting point (163°C) for the triacetate derivative of compound III by drying under vacuum at room temperature. Acetylation of flavonols removes the effect that the hydroxyl groups had on the ultraviolet spectrum with the resulting spectrum being similar to the parent flavonol (Mabry et al. 1970, p. 45). The reported spectrum of 3-methoxy-flavone had a shoulder at 320 nm and λmax at 299 and 246 nm (Geissman 1962, p. 112). The ultraviolet spectrum of the triacetate derivative (shoulder at 300,

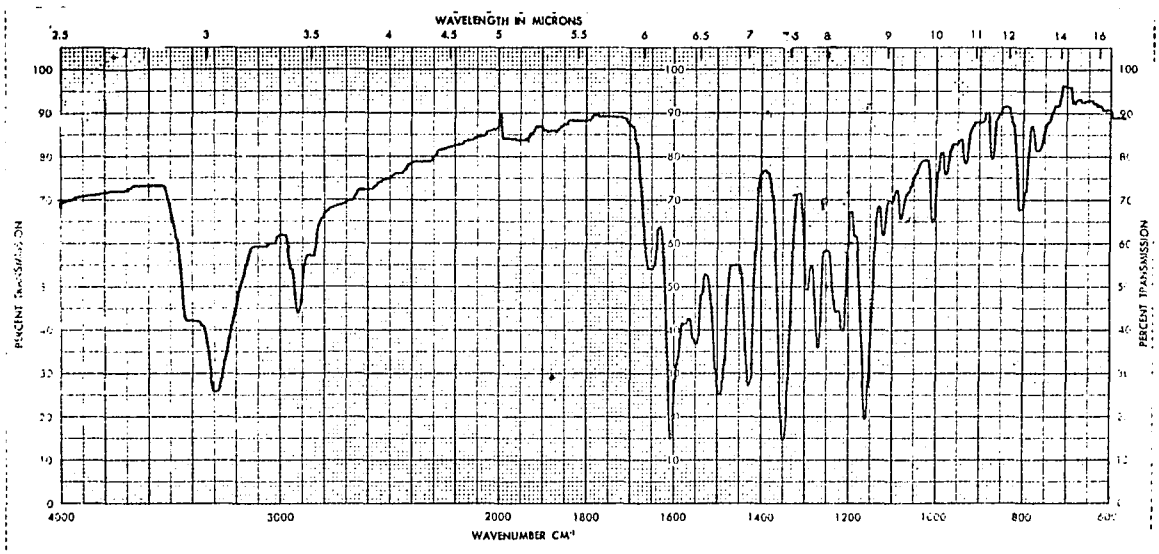


Figure 12. Infrared Spectrum of 3-O-Methylkaempferol.

λmax at 324 and 244 nm) was qualitatively similar to the reported spectrum. The infrared spectrum of the triacetate derivative was almost identical to the infrared spectrum of compound II.

Somalin

The stout white crystals of somalin (compound IV) obtained from aqueous methanol had a melting point range of 138-140°C while the crystalline needles obtained from absolute methanol had a melting point range of 197-198°C. These two crystalline forms and melting point ranges have been well established in previous reports (Karrer 1958, p. 887 [133-136°C

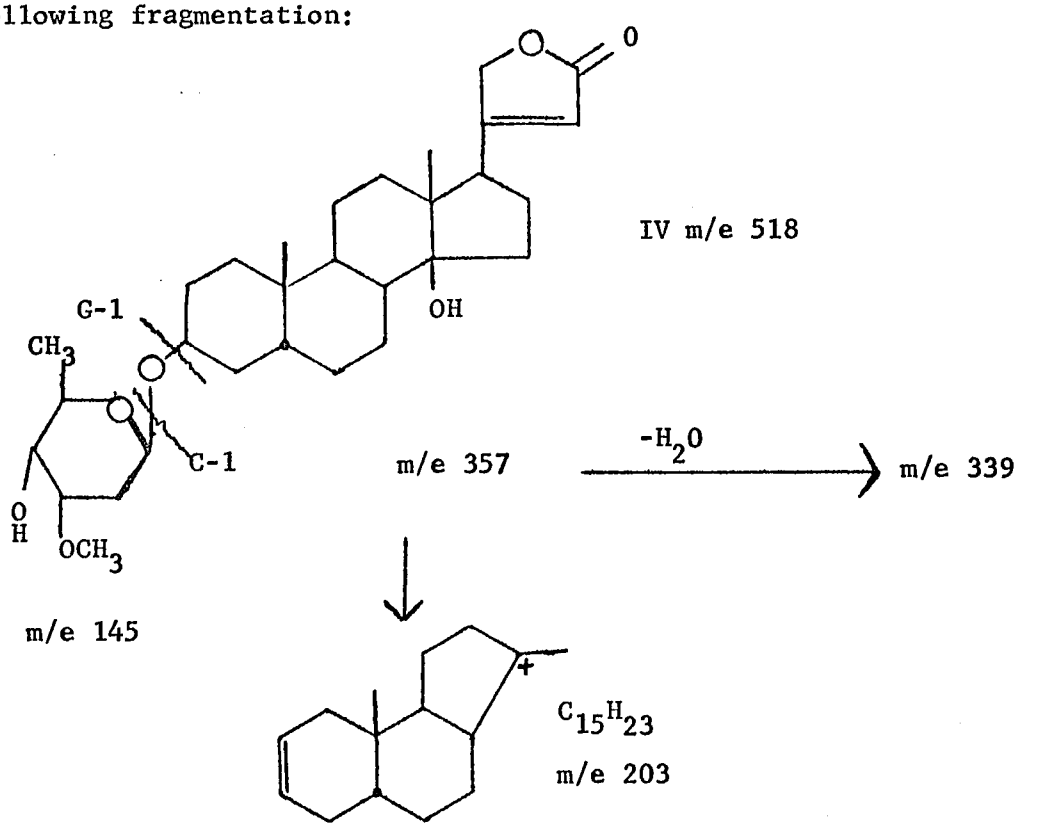
and 197-199°C]; Hartmann and Schlittler 1940, p. 550 [133-136°C and 197-198°C]; Schindler and Reichstein 1951, p. 21 [139-142°C]; Scott and Devon 1972, p. 413 [197°C]).

Carbon, Hydrogen Analysis

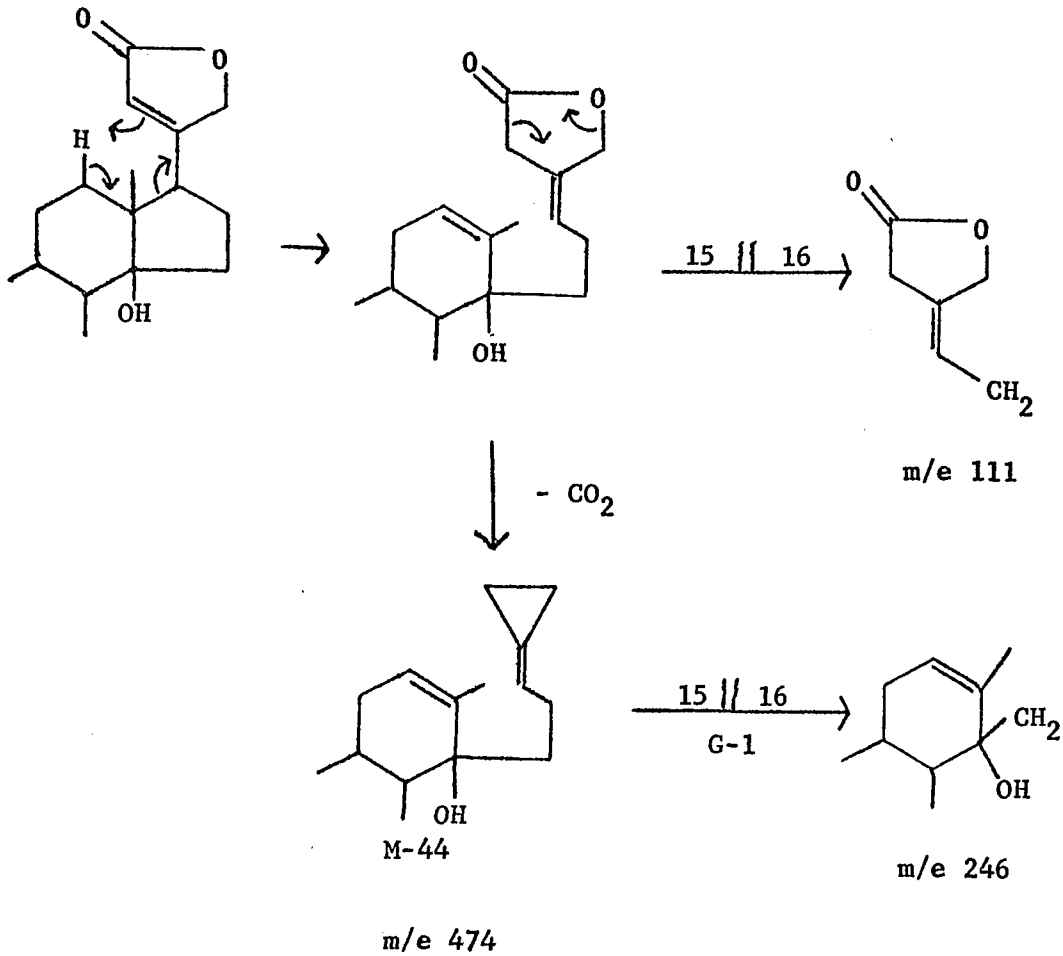
The molecular formula $C_{30}H_{46}O_7$ was substantiated by carbon, hydrogen analysis (Calculated: C, 69.47, H, 8.94; observed: C, 69.47, H, 9.11) and the mass spectrum parent peak m/e 518.

Mass Spectrum

The mass spectrum of compound IV was identical to the previously reported mass spectrum of somalin (Ardene et al. 1964, p. 1033). The base peak m/e 203 and three of the major fragments can be accounted for by the following fragmentation:



(Ardene et al. 1964, pp. 1032-1039; Spiteller 1963,p.11). Additional fragmentation that leads to an intense m/e 111 peak and accounts for an M-44 peak can be illustrated as follows:



(Budzikiewicz et al. 1964, p. 107; Spiteller-Friedman and Spiteller 1969, pp. 516-517).

Infrared Spectrum

The infrared spectrum of compound IV (Figure 13) was indicative of an alpha, beta-unsaturated-gamma-lactone ring with absorptions at 1790, 1740 and 1620 cm^{-1} (Dyer 1965, pp. 33-35; Nakanishi 1966, pp. 24, 45). Additional data confirming this structural feature was the high end absorption in the ultraviolet spectrum, λmax 220 nm (4.18); Scott 1964, p. 82).

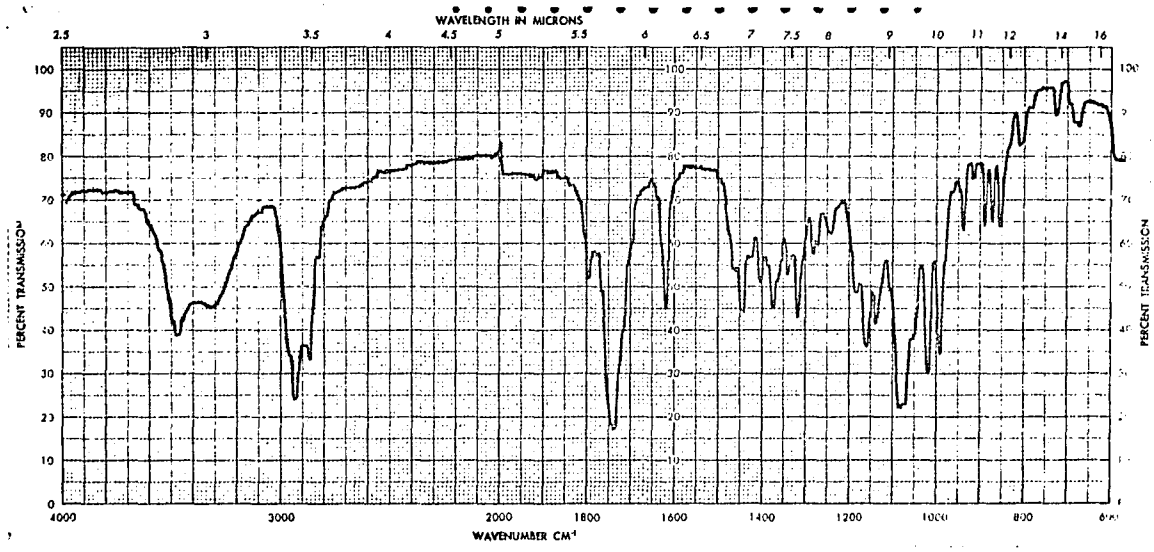


Figure 13. Infrared Spectrum of Somalin.

Nuclear Magnetic Resonance Spectrum

The nuclear magnetic resonance spectrum of compound IV (Figure 14) further verified the expected chemical shifts for the single conjugated olefinic proton (5.87 delta) and the conjugated methylene protons (4.90 delta; Bhacca and Williams 1964, p. 45). Additional data obtained from the spectrum was one glycosidic axial methoxy group at 3.43 delta (Pigman and Horton 1972, p. 309), a set of doublets centered at 4.70 delta (J = 9 cps and 1.5 cps) due to one anomeric axial proton split by the axial and equatorial protons of the two-deoxy carbohydrate unit (Pigman and Horton 1972, pp. 200-202), one equatorial C-3 proton at 4.0 delta (Bhacca and Williams 1964, pp. 77-85), two angular methyl groups

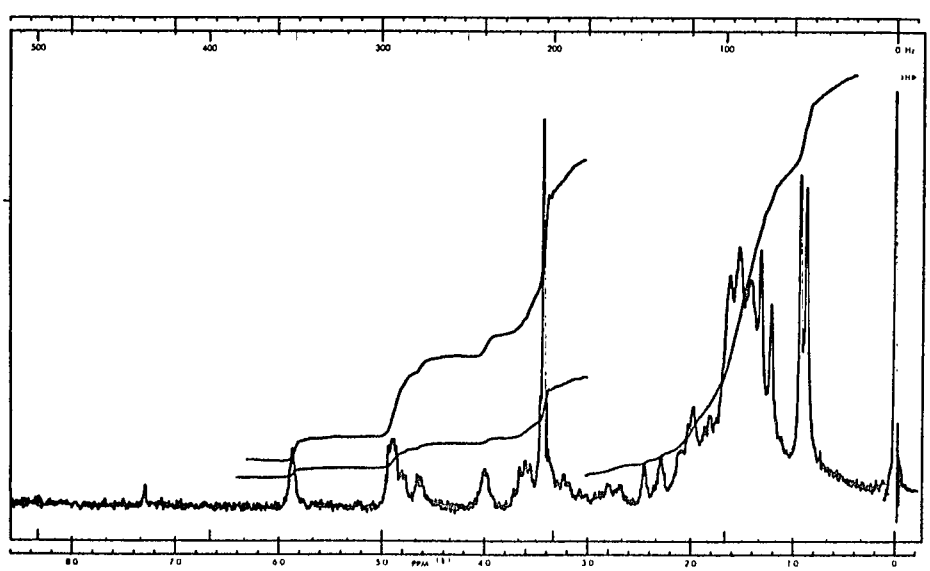


Figure 14. NMR Spectrum of Somalin.

(C-18 and C-19), 0.87 and 0.93 delta which are the exact same chemical shifts for the angular methyl groups of neriifolin obtained from Thevetia peruviana (Torrance 1975) and one freely rotating methyl group, doublet at 1.25 delta, J = 6 cps (Dyer 1965, p. 99).

Mild Acid Hydrolysis Products

After the glycosidic bond of compound IV was cleaved by mild acid hydrolysis, the crystalline genin obtained from aqueous ethanol had a melting point range of 249-250°C (reported: 250°C; Fieser and Fieser 1959, p. 754; Scott and Devon 1972, p. 413). Furthermore, no depression was observed with the mixed melting point of an authentic sample of digitoxigenin supplied by Aldrich Chemical Co., San Leandro, California, whose infrared spectrum (Figure 15)

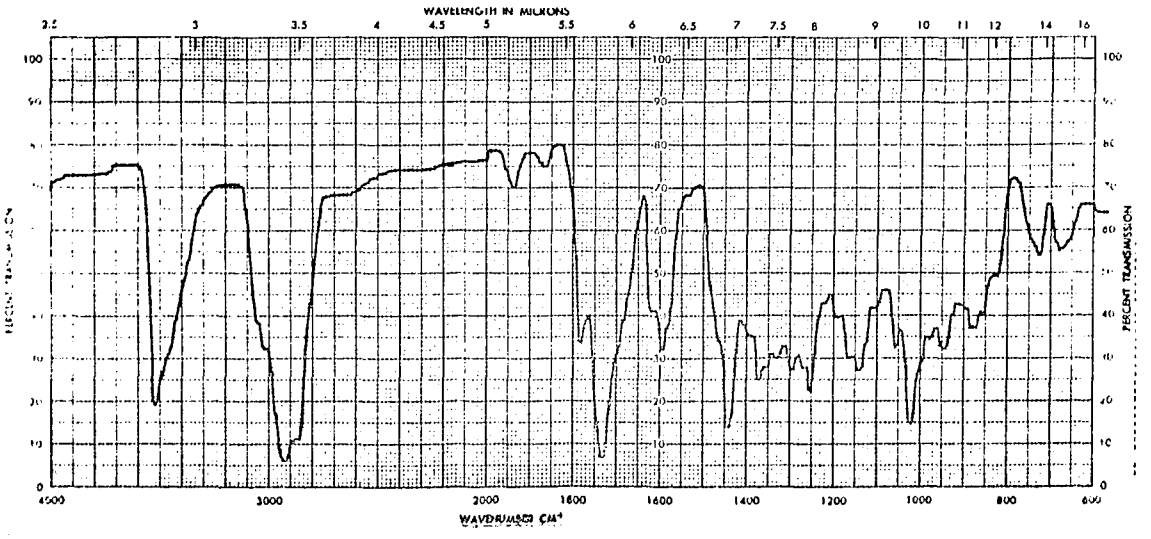


Figure 15. Infrared Spectrum of Digitoxigenin.

was superimposable with the spectrum of the genin from compound IV. The carbohydrate (D-cymarose) had a melting point range of 92-94°C (reported: 93-94°C; Merck Index 1968, p. 315), identical R_f values (0.33)in chloroform:methanol:water (188:12:1) and 0.42 in dichloromethane: methanol (95:5), and the same observed blue color after heating the chromatogram sprayed with ceric sulfate solution as did D-cymarose (mp 92-94°C) obtained from mild acid hydrolysis of an authentic sample of cymarin supplied by Sigma Chemical Co., St. Louis, Missouri.

Honghеloside A

The crystals of honghеloside A (compound V) obtained from methanol and water had a melting point range of 209-211°C; Karrer 1958, p. 901).

Carbon, Hydrogen Analysis

The molecular formula $C_{32}H_{48}O_9$ was substantiated by carbon, hydrogen analysis (calculated: C, 66.64, H, 8.39; observed: C, 66.63, H, 8.38) and the absence of a mass spectrum parent at m/e 576 coupled with the presence of an M-60 peak m/e 516 and M-44 peak m/e 532.

Mass Spectrum

A limited amount of data have been published on the fragmentation of gitoxigenin and oleandrigenin type glycosides. The base peak in the mass spectrum of compound V (Figure 16) occurs at m/e 355 due to loss of the carbohydrate fragment from the M-60 fragment, which is characteristic of the oleandrigenin type compounds (Blessington, Nakagawa and Satoh 1970, p. 215). The subsequent loss of water from the base peak fragment accounts for the intense m/e 337 peak. This later

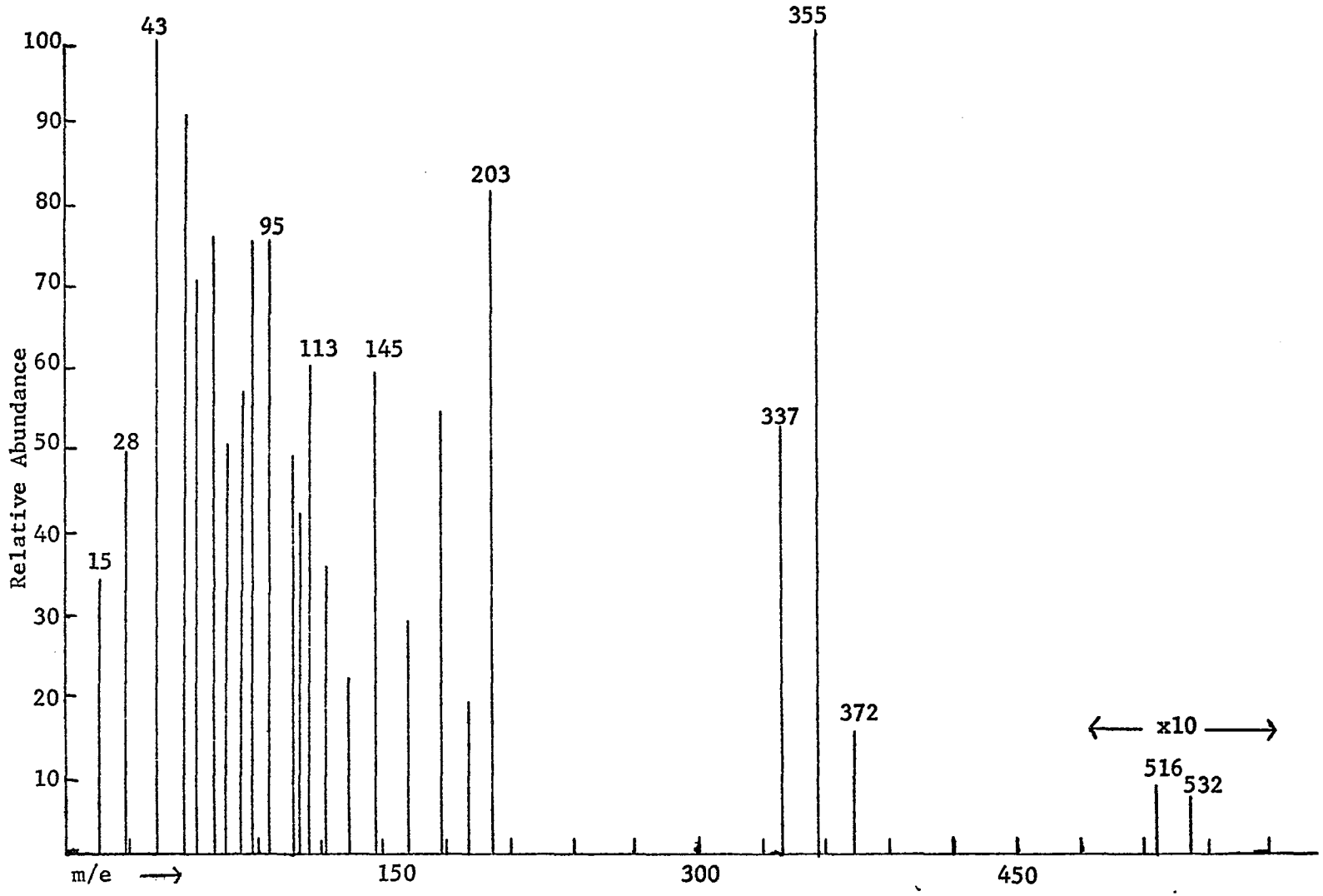
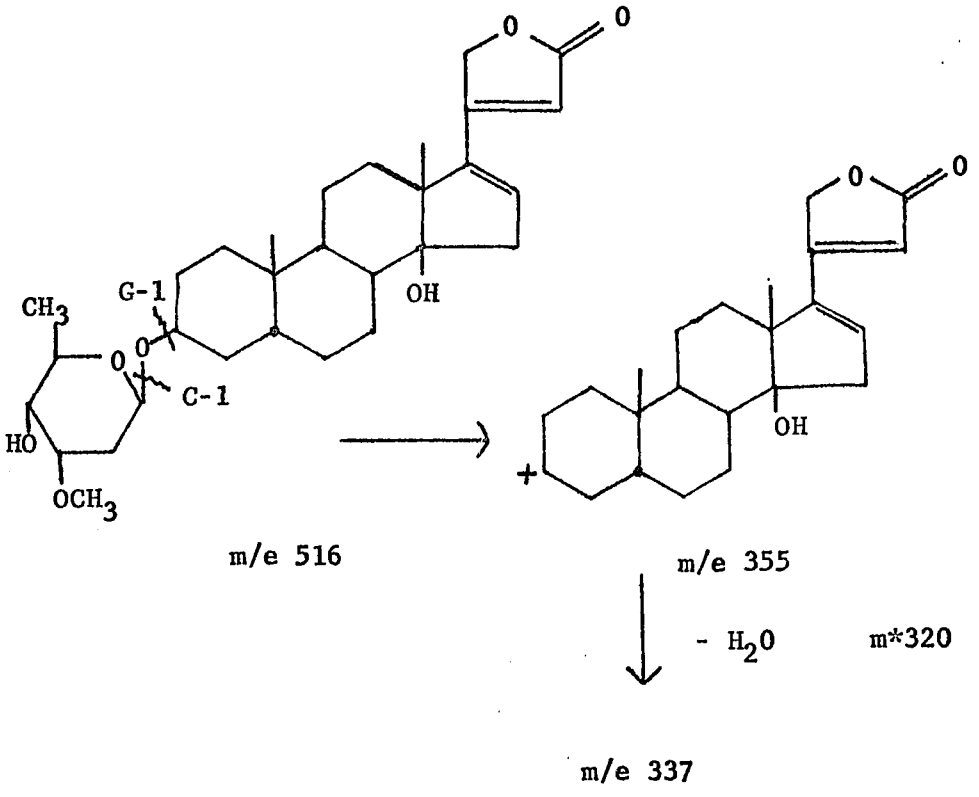
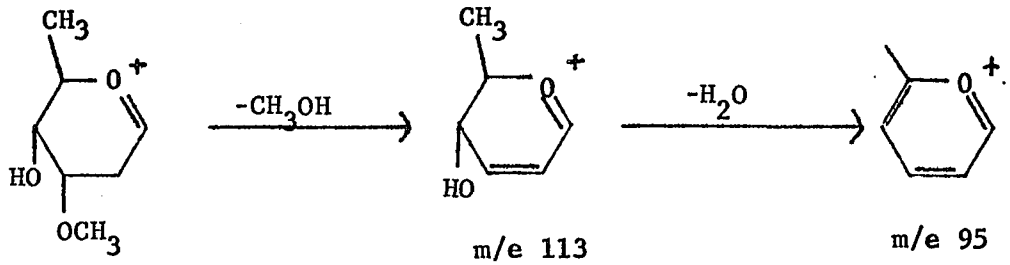


Figure 16. Mass Spectrum of Hongheloside A.

fragmentation results in the formation of a metastable peak at m/e 320 (Blessington and Morton 1970, p. 98) which is observed at increased intensity (x10 = 23%).



Further fragmentation of the carbohydrate unit could conceivably explain the appearance of intense peaks at m/e 145, 113 and 95 due to the eventual formation of a pseudo-aromatic system (Budzikiewicz et al. 1964, pp. 203-233).



m/e 145 m/e 113 m/e 95

Due to the presence of the 16-acetate functional group, the characteristic m/e 111 fragment is absent (see p. 32). Furthermore, the mass spectrum of oleandrin (sample supplied by Aldrich Chemical Co.) was qualitatively similar to the mass spectrum of compound V.

Infrared Spectrum

The infrared spectrum of compound V (Figure 17) was indicative of an alpha, beta-unsaturated-gamma-lactone ring with absorptions at 1785, 1750 and 1625 cm^{-1} (Dyer 1965, pp. 33-35; Nakanishi 1966, pp. 24, 45). The ultraviolet spectrum supplemented this data with strong end absorption λmax 220 nm (log ε = 4.12); (reported 217 nm [log ε = 4.14]; Scott 1964, p. 417). An additional absorbance at 1245 cm^{-1} was indicative of the acetate ester (Nakanishi 1966, p. 45).

Nuclear Magnetic Resonance Spectrum

The nuclear magnetic resonance spectrum of compound V (Figure 18) and oleandrin were very similar. The conjugated olefinic proton appeared at 5.93 delta, the conjugated methylene protons at 4.90 delta, the anomeric axial proton, the 3-equatorial proton and the axial methoxy

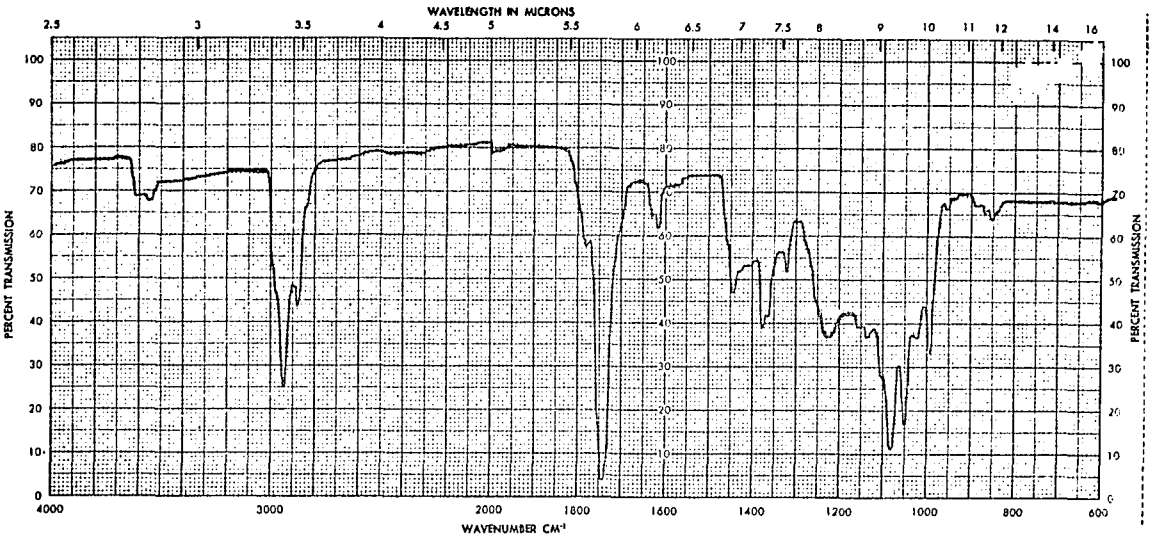


Figure 17. Infrared Spectrum of Hongheloside A.

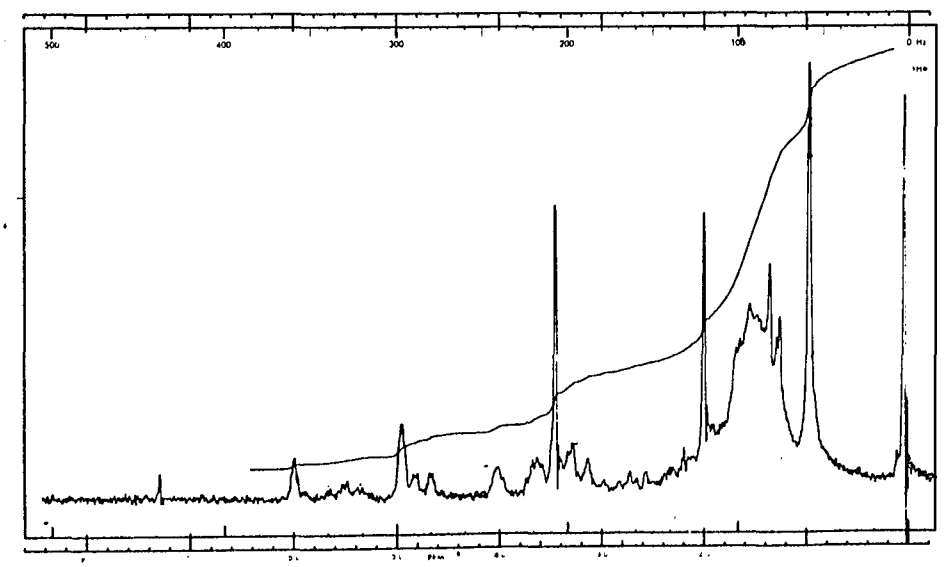


Figure 18. NMR Spectrum of Hongheloside A.

protons had the same appearance and chemical shift as in the spectrum of compound IV (see p. 34), the acetate ester protons appeared at 1.98 delta and the adjacent 16-proton appears as a broad multiplet (18 cps) centered at 5.45 delta (Bhacca and Williams 1964, pp. 77-85). As a result of the acetate functional group at C-16, both angular methyl groups appear as a singlet at 0.93 delta, which is identical with the results from oleandrin. As with compound IV, the freely rotating methyl group on D-cymarose appears as a doublet at 1.25 delta (J = 6 cps).

Mild Acid Hydrolysis Products

The carbohydrate moiety recovered after mild acid hydrolysis of the deacetylated derivative of compound V was identical to the data reported for D-cymarose (see p. 36), with a melting point range of 92-94°C. The genin had a melting point range of 225-227°C (reported: 224°C, Scott and Devon 1972, p. 417 [225°C]; Fieser and Fieser 1959, p. 756 [234°C]; Merck Index 1968, p. 490). The infrared spectrum (Figure 19) of an authentic sample of gitoxigenin from K and K Laboratories, Irvine, California, (mp. 228-232°C) was superimposable with that of the genin from compound V's deacetylated derivative.

16-Acetylstrospeside

The colorless platelets of 16-acetylstrospeside (compound VI) had a melting point range of 208-210°C which was reduced to 189-190°C after drying under vacuum.

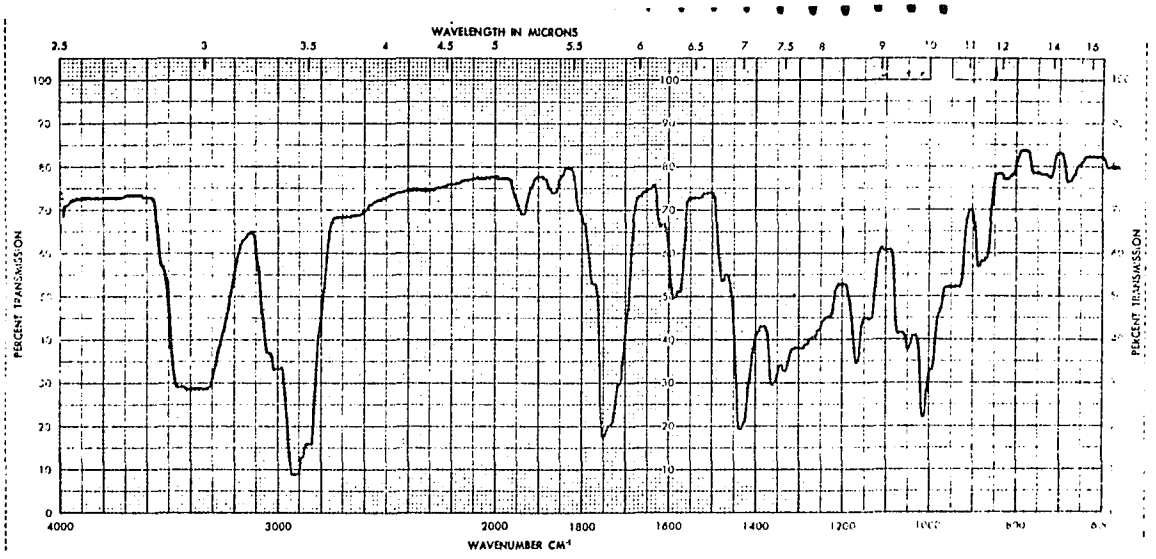


Figure 19. Infrared Spectrum of Gitoxigenin.

Mass Spectrum

The mass spectrum of compound VI (Figure 20) exhibited the characteristic base peak m/e 355 followed by an intense m/e 337 peak due to loss of water coupled with the corresponding metastable peak m/e 320 (x10 = 18%; Blessington et al. 1970, p. 215; Blessington and Morton 1970, p. 98). Comparing this spectrum with the mass spectrum of compound V, the decreased intensity or absence of mass peaks m/e 145, 113 and 95 can be attributed to the additional oxygen atom at the two position in the carbohydrate moiety and therefore, a decreased intensity in the specific

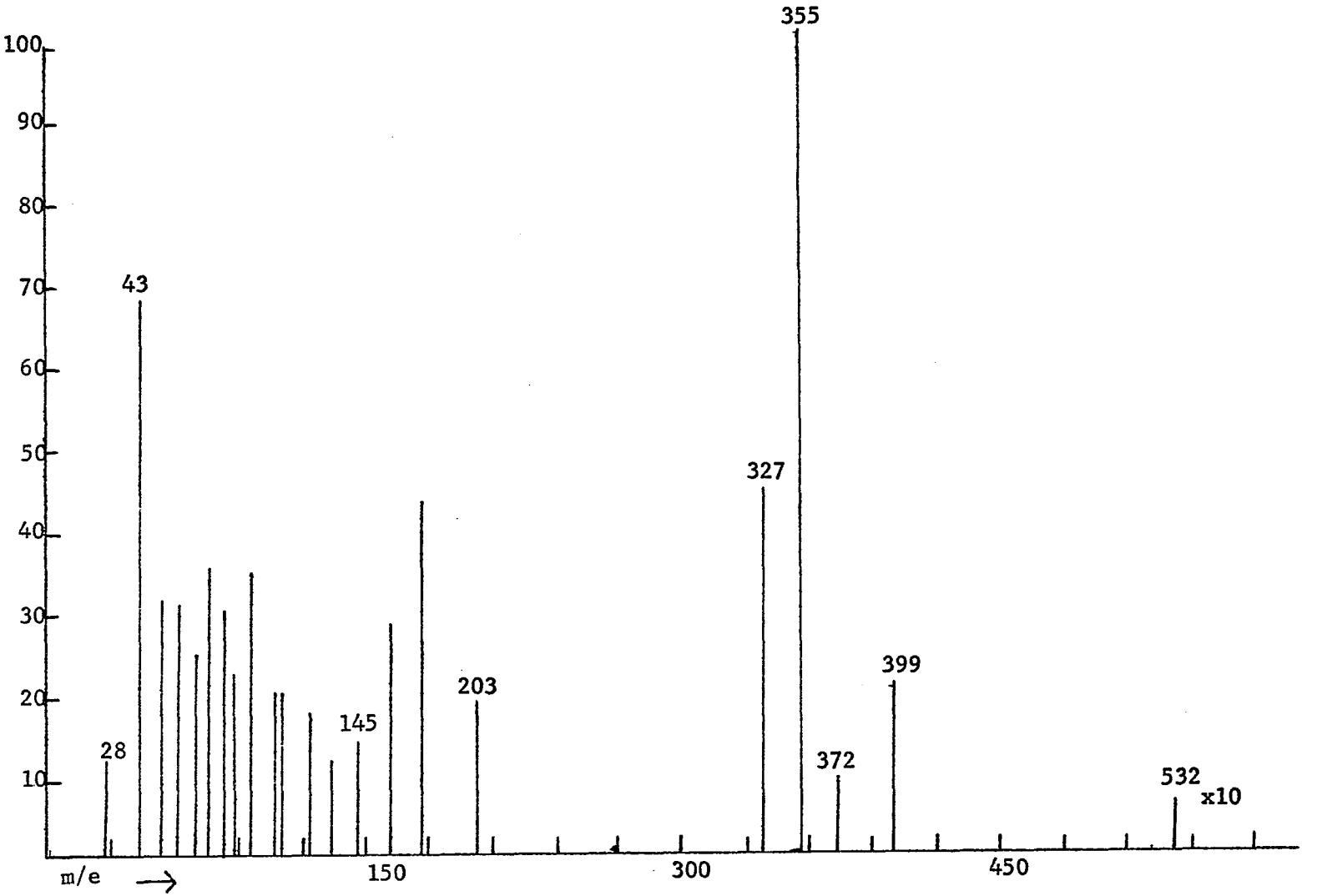
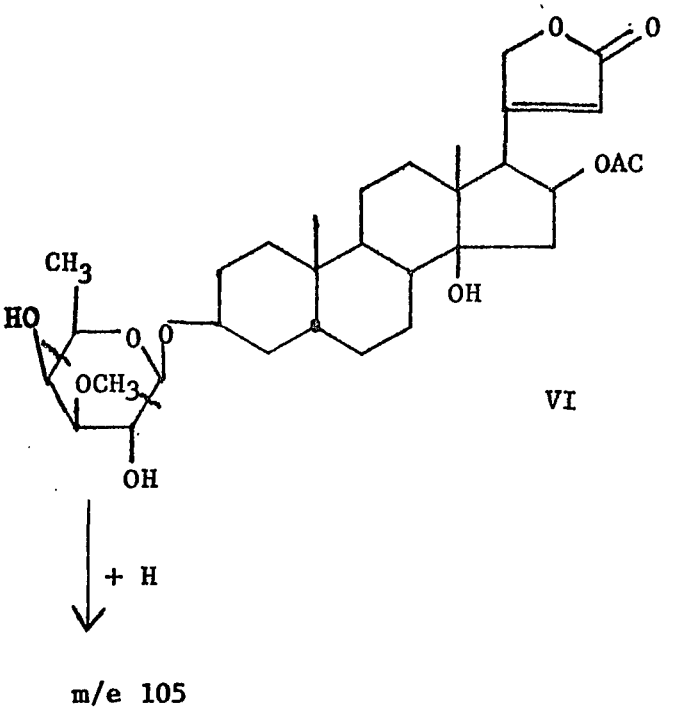


Figure 20. Mass Spectrum of 16-Acetylstrospeside.

fragments that may form. An example of this more complex type of fragmentation has been reported (Brown et al. 1971, p. 582).



VI

m/e 105

Infrared Spectrum

In agreement with the previous two cardenolide glycosides, the infrared spectrum of compound VI (Figure 21) was indicative of the alpha, beta-unsaturated-gamma-lactone ring (1785, 1745 and 1620 cm^{-1}). The additional absorbance at 1245 cm^{-1} was indicative of the acetate ester (Nakanishi 1966, p. 45). The high end absorption in the ultraviolet spectrum (λmax 220 nm, log ϵ = 4.31) was additional verification (Scott 1964, p. 82).

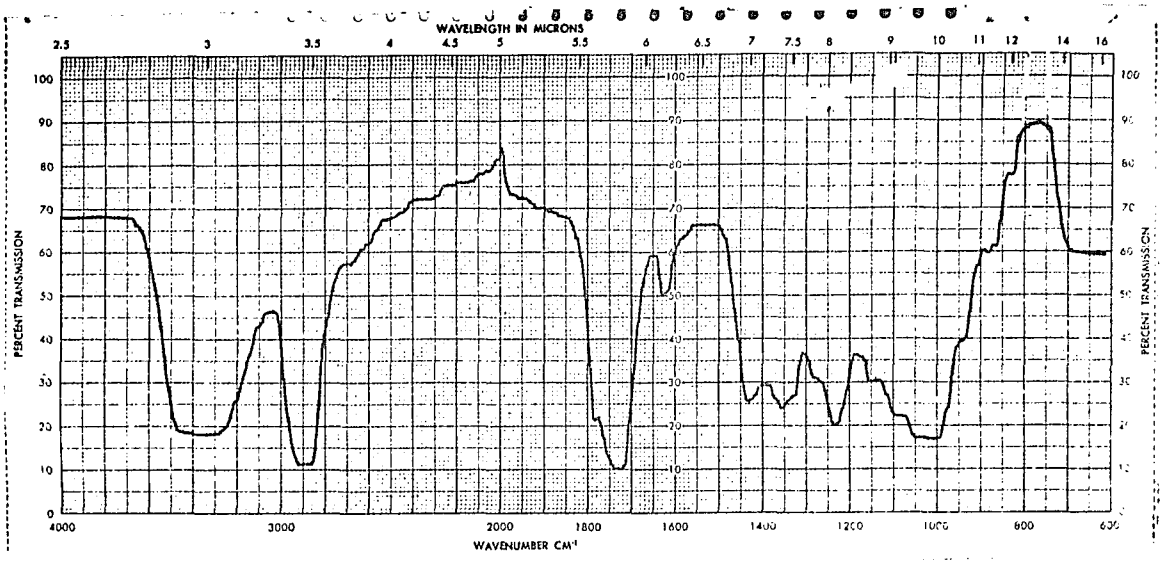


Figure 21. Infrared Spectrum of 16-Acetylstrospeside.

Nuclear Magnetic Resonance Spectrum

The nuclear magnetic resonance spectrum of compound VI (Figure 22) had the expected peaks at 5.93 delta for conjugated olefinic proton, 4.95 delta for conjugated methylene protons, and 1.98 delta for the acetate protons. The adjacent C-16 proton appears as a broad multiplet (18 cps) centered at 5.50 delta, the equatorial glycosidic methoxy group appears at 3.60 delta (Pigman and Horton 1972, p. 309), the axial anomeric proton has shifted upfield as expected (Pigman and Horton 1972, p. 200-202), the C-3 equatorial proton appears at 4.00 delta, the freely rotating methyl at 1.27 delta (J = 6 cps) and both angular methyl groups

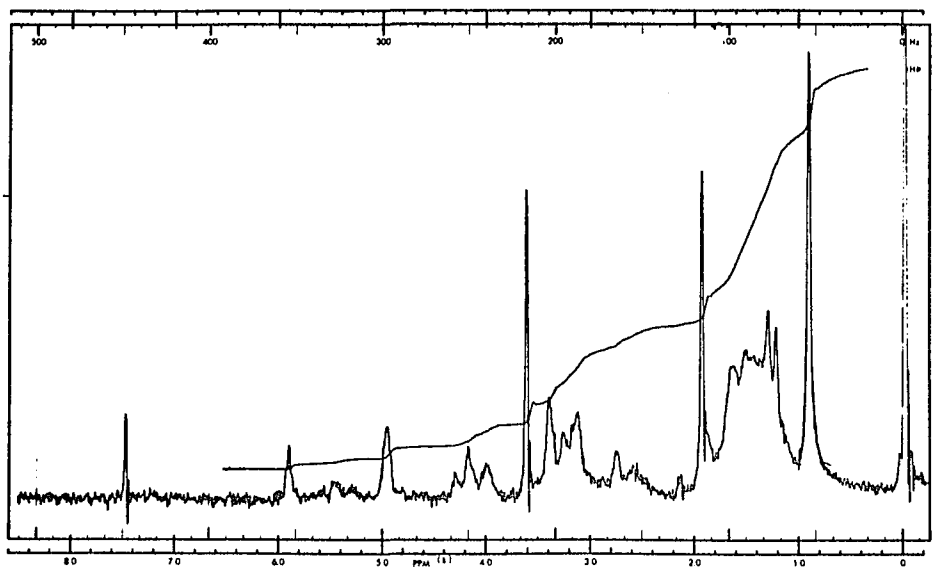


Figure 22. NMR Spectrum of 16-Acetylstrospeside.

appear as a singlet at 0.93 delta, consistent for oleandrigenin type glycosides.

Desacetyl Derivative of 16-Acetylstrospeside

Compound VI was deacetylated to produce the known compound strospeside. The crystals obtained from methanol and water had a melting point range of 252-253°C (reported: 248-255°C [Karrer 1958, p. 896]; 252°C [Scott and Devon 1972, p. 417]).

16-Anhydro Derivative of 16-Acetylstrospeside

The crystals obtained from methanol and water of the 16-anhydro derivative of compound VI had a melting point range of 240-243°C (reported: 242-246°C [Karrer 1958, p. 899]). Furthermore, the ultraviolet spectrum had the characteristic absorption: λmax 270 nm, log ε = 4.25 (Hunger and Reichstein 1950b, p. 1995).

Honghelin

The hydrate crystals of honghelin (compound VII) obtained from methanol and water had a melting point range of 135-137°C (reported: 133-136°C; Karrer 1958, p. 889).

Infrared Spectrum

The infrared spectrum of compound VII (Figure 23) was indicative of a cardenolide glycoside with absorption at 1780, 1740 and 1620 cm^{-1} for the alpha, beta-unsaturated-gamma-lactone ring (Dyer 1965, pp. 33-35; Nakanishi 1966, pp. 24, 45). The high end absorption in the ultraviolet spectrum was observed at λmax 220 nm (log ε = 4.25).

Nuclear Magnetic Resonance Spectrum

The nuclear magnetic resonance spectrum of compound VII (Figure 24) had the expected peaks at 5.83 delta, one conjugated olefinic proton; 4.90 delta, conjugated methylene protons; 3.60 delta, one equatorial glycosidic methoxy group (Pigman and Horton 1972, p. 309); 4.00 delta, one equatorial C-3 proton; one axial anomeric proton shifted upfield (Pigman and Horton 1972, pp. 200-202); and C-18, C-19 angular

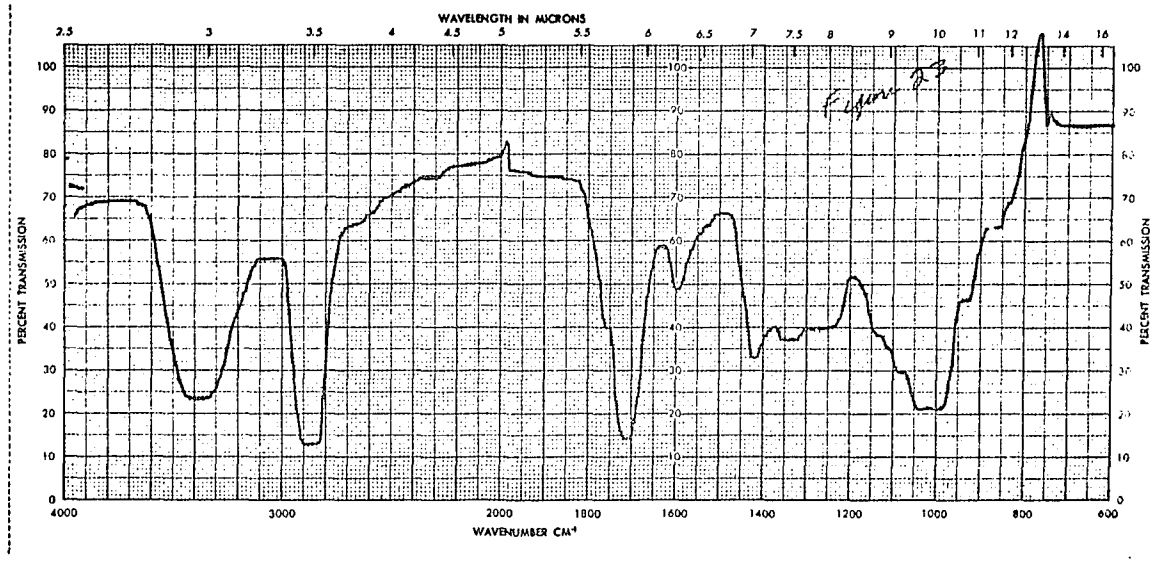


Figure 23. Infrared Spectrum of Honghelin.

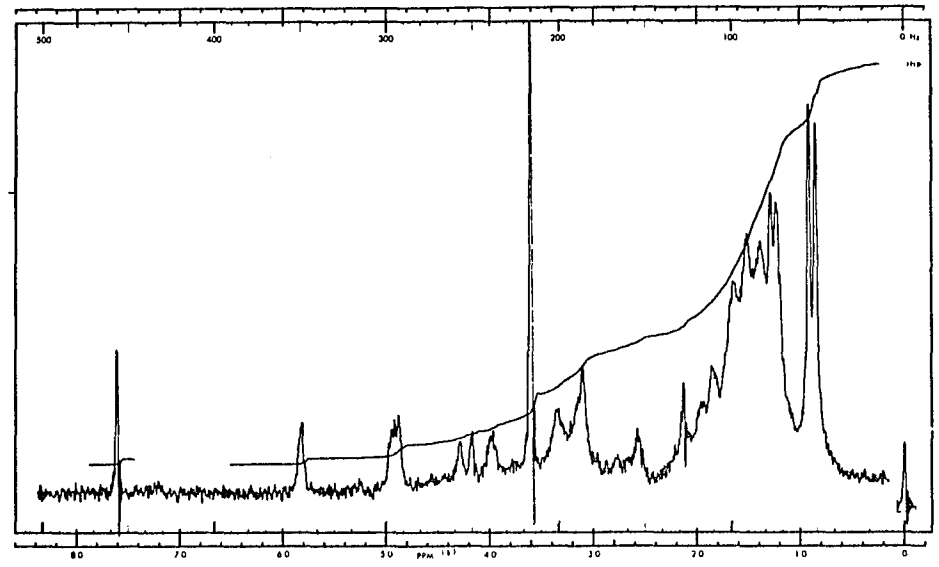
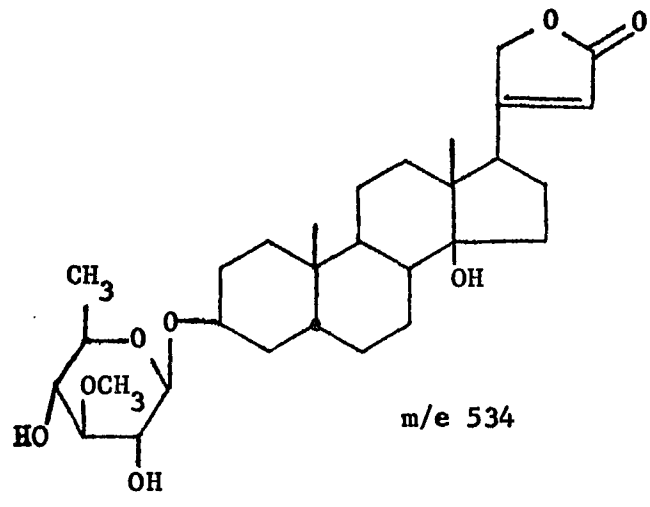


Figure 24. NMR Spectrum of Honghelin.

methyl groups at 0.87 and 0.93 delta, consistent with digitoxigenin glycosides (e.g., somalin and neriifolin).

Mass Spectrum

The mass spectrum of compound VII was identical with the mass spectrum of neriifolin (Brown et al. 1971, p. 582), which contains L-thevetose at the 3-beta position while compound VII contains D-thevetose at the 3-beta position. The parent peak is m/e 534 with the next observable peak m/e 516 due to loss of water. The characteristic peaks at m/e 357, 339 and 203 predominate and are due to fragmentations of the genin (see p. 38).



VII

Diacetate Derivative of Honghelin

The diacetate derivative after recrystallization from methanol and water had a melting point range of 218-220°C (reported: 217-220°C, Reyle and Reichstein 1952, p. 204). The molecular formula $C_{34}H_{50}O_{12}$ was confirmed by carbon, hydrogen analysis (calculated: C, 66.00, H, 8.14; observed: C, 66.29, H, 8.20). The addition of two acetate groups was confirmed by the presence of the two methyl singlets at 2.07 and 2.10 delta in the nuclear magnetic resonance spectrum of the diacetate derivative (Figure 25). Additional information obtained from the spectrum was the usual downfield shift of the C-2 and C-4 carbohydrate protons which overlap with the conjugated methylene protons but are spread out over 28 cps, indicative of two axial protons (Pigman and Horton 1972, pp. 200-202). Furthermore, the C-1 axial anomeric proton appears as a doublet at 4.40 delta, J = 8 cps; the freely rotating methyl group appears as a doublet at 1.23 delta, J = 6 cps; and one of the angular methyl groups has shifted slightly (0.87 to 0.88 delta).

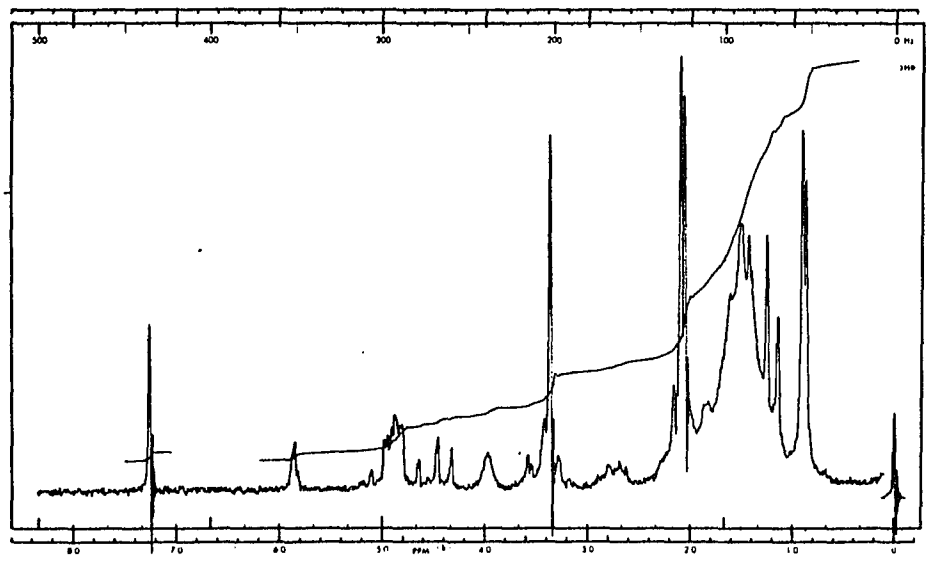


Figure 25. NMR Spectrum of Honghelin Diacetate.

CHAPTER 4

EXPERIMENTAL

The first part of this chapter deals with the procedures and equipment used to concentrate the cytotoxic activity of the plant primarily within one fraction. The remainder of the chapter has been divided into three sections. The first section deals with the chromatographic techniques used in the isolation of the plant constituents. The second section deals with the procedures and various equipment used to obtain the necessary physical data. The final section deals with the chemical procedures and methods used in the preparation of the various derivatives.

Grinding of the Plant

The stems, leaves and flowers of the plant were ground to a coarse powder in a Wiley Mill (Arthur H. Thomas Co., Philadelphia, Pennsylvania) equipped with a 3-mm sieve.

Extraction of Plant

A four liter capacity Lloyd-type extractor (Brighton Copper Works, Cincinnati, Ohio) was used to defat a 15-kg sample of the powder with sufficient petroleum ether until the solvent flowing from the extractor was colorless. Subsequently, the defatted marc was extracted with 95 percent ethanol to yield 1.2 kg of air dried extract.

Solvent Partitioning and Extraction

The results of the solvent partitioning and extraction are summarized in Figure 1 (p.5). The partitioning was accomplished by dissolving a 400-gm sample of the ethanol extract in an equal mixture (3 liter) of chloroform and water. This mixture was transferred into a four-liter separatory funnel, shaken vigorously, the chloroform layer removed, and an additional 1.5 liter of chloroform added to the funnel. This procedure was repeated until the chloroform layer was almost colorless. The combined chloroform fractions were extracted once with water and air dried.

The solvent extractions were all performed in the same manner. The fraction being extracted was suspended in the solvent by stirring with an automatic stirrer (Electrical Appliances, Inc.), filtered and the procedure repeated until the filtrate was almost colorless. All fractions obtained were air dried and subsequently stored in a freezer at $-10^{o}C$.

Chromatography

Brinkman Instruments Inc., Westbury, New York, was distributor of the silica gel-G, silica gel-60, and silica gel-PF_{254} (Merck).

Thin Layer

The plates were prepared with an automatic plate maker (Camag, Homburger-strasse 24, Muttenz, Switzerland) distributed by Gelman Instrument Co., Ann Arbor, Michigan. The plates were covered with a 0.3-mm layer of silica gel-G and activated by heating for one hour or more in an

oven kept at 110°C. The plates were developed in the various solvent systems (see Chapter 2), air dried, observed under ultraviolet (254 and 350 mm Universal UV Lamp, Gelman, Camag, Switzerland), sprayed with ceric sulfate solution (20 gm of ceric sulfate plus 56 ml of concentrated sulfuric acid plus one liter of water), warmed gradually with a heat gun (Master Appliance Corporation, Racine, Wisconsin), observed under ultraviolet light and then charred in an oven at 110°C.

Silica Gel-G Columns

Fractionation of ether-soluble fraction was carried out on two successive silica gel-G columns. A glass column (7 cm by 130 cm) was packed with silica gel-G in the following manner: a cotton plug was introduced into the stem of the column, immediately above the stopcock which was left open; after introducing another cotton plug on the bottom of the column, a small amount of dry silica gel-G was poured on top of the cotton; then one liter of solvent system (petroleum ether:ether: chloroform (1:3:1) was poured into the column and the drop rate was adjusted to a steady stream; a slurry of 1.5 kg of silica gel-G and the solvent system was poured into the column; after the addition was completed, the column was filled with the solvent system and the drop rate adjusted to one every several seconds. The column was left standing overnight to insure a homogeneous firmly packed column. The next day a dried 50-gm sample of the extract (which had been dissolved in chloroform and absorbed on 60 gm of silica gel-G) was introduced onto the column (7 cm x 81 cm) with only a few cm of solvent system above the silica gel-G level, which was subsequently covered with 2-3 cm of dry

silica gel-G. Then the column was refilled with the solvent system and the drop rate adjusted to one drop per second. An automatic fraction collector (Buchler Instruments, Inc., Fort Lee, New Jersey) was used to separate the fractions (25 ml per tube).

The silica gel-G column used to isolate honghelosides A was packed in the same manner as before. However, the column size was 4 cm by 42 cm (250 gm of silica gel-G for 2.5 gm of sample) and the solvent system was dichloromethane:methanol (96:4).

Preparative Plate Thin Layer

The preparative plates were prepared in the same manner as the thin layer plates with the only difference being 1.5 mm thickness of silica gel-PF_{254} instead of 0.3 mm of silica gel-G. The sample to be separated was applied with a Camag chromatographer at concentrations of 50-60 mg per plate. After several developments in the appropriate solvent system, the plates were observed under ultraviolet light (254 mm) and the far left side was sprayed with ceric sulfate solution and gently heated until the spots appeared. Then the appropriate section was removed with a razor blade, the silica gel-PF_{254} mixed with an equal mixture of chloroform and methanol, filtered and the solvent removed under vacuum to yield the desired compound.

Silica Gel-60 Columns

The two silica gel-60 columns were packed in the same manner as described for the silica gel-G columns. The columns used were 6 cm by 120 cm glass columns packed with silica gel-60 to a height of 70 cm

(1.2 kg) with 25 gm of sample for each column. The solvent system was chloroform:methanol:water (188:12:1).

Physical Data

Unless otherwise indicated, the physical data was collected at the Department of Chemistry and College of Pharmacy, University of Arizona, Tucson, Arizona.

Melting Point Determination

A Kofler block was used to determine the uncorrected melting points.

Carbon, Hydrogen Analysis

The carbon, hydrogen analyses were determined by Chemalytics, Inc., 2330 South Industrial Park Drive, Tempe, Arizona. All samples sent for analysis were previously dried under vacuum at 111°C for at least 24 hours and weighed 5-8 mg.

Infrared Spectroscopy

Most of the infrared spectra were obtained from the potassium bromide pellet of the compound on a Beckman IR-33. Compound V and the derivatives of compound I were run in spectra grade chloroform (0.5 mm KBr cells).

Ultraviolet Spectroscopy

The ultraviolet spectra were run in spectra grade methanol using a Beckman DB-G Grating Spectrophotometer (Beckman, Fullerton, California).

The microanalytical ultraviolet spectra of the flavonols were obtained according to previously reported procedures (Mabry et al. 1970, pp. 35-39).

Nuclear Magnetic Spectroscopy

The Varian T-60 (Varian Associates, 611 Hansen Way, Palo Alto, California, 94303) was utilized for all nuclear magnetic spectra, which were run in deuterated solvents in 20 percent concentration using tetramethylsilane as an internal standard. Compound I was run in deuterated pyridine, derivatives of compound I and compounds IV and V were run in deuterated chloroform, compounds II and III were run in deuterated dimethylsulfoxide, and compounds VI and VII were run in deuterated chloroform plus two drops of deuterated dimethylsulfoxide.

Mass Spectrometry

The mass spectra were obtained on a Hitachi, Perkin-Elmer RMU-6E double focusing mass spectrometer with an all glass inlet system (Perkin-Elmer Corp., 1625 East Edinger, Santa Ana, California).

Preparation of Derivatives

Acetylation of Dihydroifflaionic Acid

Compound I (300 mg) was dissolved in 3 ml of freshly distilled pyridine. Acetic anhydride (6 ml) was added and solution remained standing at room temperature for 48 hours. The reaction mixture was poured into a beaker of ice water and stirred briskly. The resultant precipitate was filtered, washed several times with water and transferred

to a desiccator containing potassium hydroxide pellets. After standing for 24 hours a quantitative yield of the acetate had been recovered.

Preparation of Diazomethane

An ice-salt bath was used to cool a solution of 4 ml of 40 percent aqueous potassium hydroxide and 20 ml of ether to 0°C. During a period of 15 minutes, 1.5 gm of N-methyl-N'-nitro-N-nitrosoguanidine was added with swirling to the previous solution. The reaction mixture was transferred to a precooled separatory funnel, aqueous layer removed and the etheral diazomethane was transferred to a 50 ml dry precooled flask. After the addition of a few potassium hydroxide pellets the flask was tightly stoppered and refrigerated overnight.

Methyl Ester of Dihydroifflaionic Acid

Compound I (50 mg) was dissolved in 50 ml of dry ether, cooled to 0°C in an ice-salt bath and 7 ml of the cold etheral diazomethane was added over a period of ten minutes. After the reaction mixture was left standing at room temperature for two hours the solvent was evaporated in a hood and the resulting crude product was crystallized from methanol.

Methyl Ester of Acetyldihydroifflaionic Acid

The acetate derivative of compound I (100 mg) was dissolved in 40 ml of dry ether and cooled to 0°C in an ice-salt bath. The remaining 13 ml of ethereal diazomethane was added over a period of 15 minutes. After the reaction mixture was left standing at room temperature for two hours the ether was evaporated in a hood. The previous three procedures were modifications of a known procedure (Lane 1969).

The resulting residue was purified by preparative plates with hexane: benzene:ethyl acetate (6:3:1) as the developing solvent system.

Acetylation of 3,3'-Di-O-methylquercetin

Compound II (30 mg) was dissolved in one ml of pyridine and two ml of acetic anhydride, left standing at room temperature for 48 hours, poured into a separatory funnel containing 20 ml of ice water, and extracted three times with 20 ml of ether. The ether solution was washed with 30 ml of ten percent hydrochloric acid, several times with water, with five percent aqueous sodium bicarbonate to hydrolyze the unreacted acetic anhydride, and with water until neutral pH was obtained. After drying the ether solution with anhydrous magnesium sulfate, the ether was evaporated off under vacuum and the residue stored over potassium hydroxide pellets in a desiccator for 24 hours. The triacetate derivative was purified on preparative plates with benzene:ether (8:2) as the developing solvent system.

Acetylation of 3-O-Methylkaempferol

Compound III (30 mg) was converted into the triacetate derivative following the exact same procedure as described for compound II.

Mild Acid Hydrolysis of Somalin

Compound IV (50 mg) was dissolved in 50 ml of tetrahydrofuran (freshly distilled over potassium hydroxide pellets) and flushed with nitrogen. A solution (1 ml) of 0.1 ml 62 percent perchloric acid diluted to ten ml with tetrahydrofuran was added to the glycoside solution in a nitrogen atmosphere. The reaction mixture was heated in a constant

temperature bath at 55°C for 30 minutes, cooled, and quenched with 25 ml of water and ten ml of 0.01M ammonium hydroxide. After removing the tetrahydrofuran under vacuum the aqueous suspension was extracted with chloroform. The water phase was evaporated to dryness, extracted with acetone, filtered, and triturated in ether to yield crystalline D-cymarose. The chloroform phase was dried with anhydrous magnesium sulfate, filtered, concentrated to one ml and purified on a preparative plate with dichloromethane:methanol (95:5) as the developing solvent system. This procedure was a modification of a previously reported kinetic study on the acid hydrolysis of cardiac glycosides (Frey and Jacobsohn 1970, pp. 78-85).

Deacetylation of Honghelosides A

Compound V (100 mg) was dissolved in 20 ml of methanol. Then potassium bicarbonate (100 mg) was dissolved in four ml of water added to the methanol solution and the reaction mixture was stirred for three days at room temperature. The reaction mixture was evaporated to dryness, extracted with chloroform, filtered and purified on preparative plates with dichloromethane:methanol (96:4) as the developing solvent system.

Mild Acid Hydrolysis of Desacetylhonghelosides A

The same procedure for the hydrolysis of somalin was utilized for the hydrolysis of desacetylhonghelosides A. Furthermore, the same procedure was also utilized for the hydrolysis of cymarin.

Deacetylation of 16-Acetylstrospeside

The same procedure as the procedure for the deacetylation of compound V was utilized for the deacetylation of compound VI. However, strospeside was purified on preparative plates with benzene:ethyl acetate: acetone:ethanol (4:3:2:1) as the developing solvent system.

16-Anhydrostrospeside

The 16-anhydro derivative of compound VI was prepared by absorbing the compound (30 mg) on basic aluminum oxide, which was then introduced on top of a small aluminum oxide column (1.5 cm by 15 cm). The column was eluted with benzene:methanol (9:1) until the compound was displaced somewhere beyond the origin, the stopcock was closed, and the wet column remained at room temperature for one week. Then the column was eluted clean with chloroform:methanol (1:1). The resulting compound was crystallized from methanol and water. After two subsequent recrystallizations a relatively pure derivative was obtained as indicated by the absence of absorption in the ultraviolet spectrum in the 220 nm region.

Acetylation of Honghelin

Compound VII (100 mg) was dissolved in two ml of pyridine and four ml of acetic anhydride and warmed gently on a steam bath until the thin layer chromatogram exhibited no observable compound VII and only one spot when developed in benzene:ethyl acetate:ether (1:1:1). After three hours the reaction mixture was poured into ice water, filtered, washed with water, left standing over potassium hydroxide pellets in a desiccator for 48 hours and then crystallized from methanol and water.

CHAPTER 5

SUMMARY AND CONCLUSION

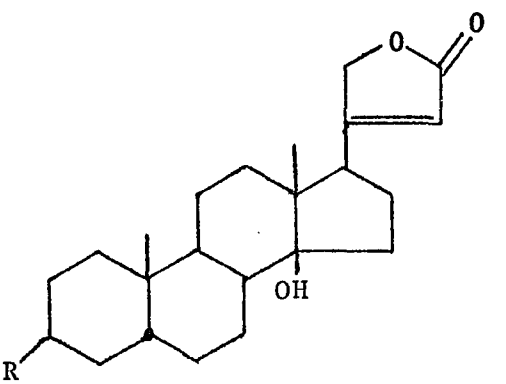
Seven compounds were isolated from the ether soluble fraction of Adenium obesum, family Apocynaceae. Four of the seven compounds were identified as the following cardenolide glycosides: somalin, honghe loside A, 16-acetylstrospeside, and honghelin. Of the remaining three, one was dihydroiffl aionic acid and the other two were flavonols: 3-O-methyl-kaempferol and 3,3'-di-O-methylquercetin.

Dihydroiffl aionic acid and 3-O-methylkaempferol were inactive in the 9KB test system. The 9KB cytotoxic activity (micrograms per milliliter) of the remaining five compounds was as follows: 3.08 for 3,3'-di-O-methylquercetin, 0.035 for honghe loside A and 16-acetylstrospeside, 0.02 for honghelin and less than 0.01 for somalin. Additional material is being collected to test for anti-tumor activity in the 3PS test system.

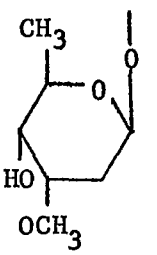
APPENDIX A

MOLECULAR STRUCTURES OF CARDENOLIDE GLYCOSIDES
ISOLATED FROM ADENIUM SPECIES

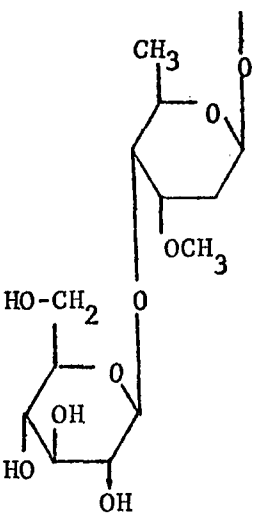
Digitoxigenin: R = OH



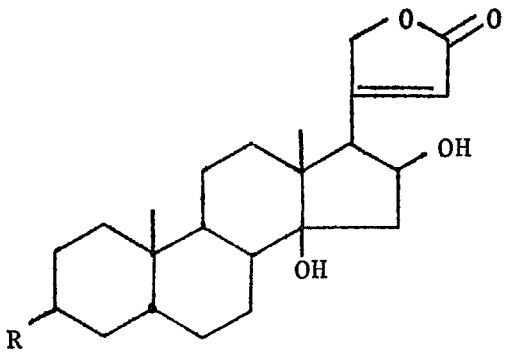
Somalin: R = D-cymarose



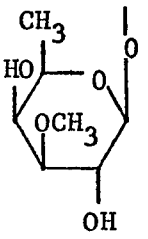
Echujin: R =



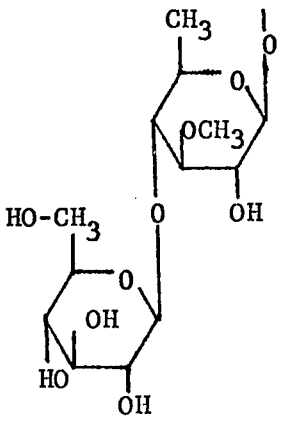
Gitoxigenin: R = OH



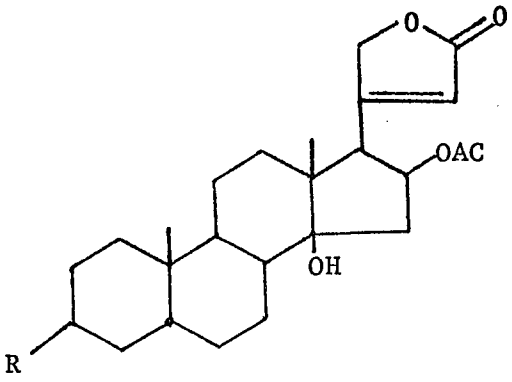
Strospeside: R = D-digitalose



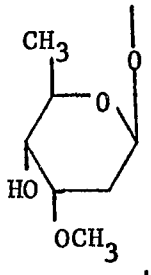
Digitalinum verum: R =



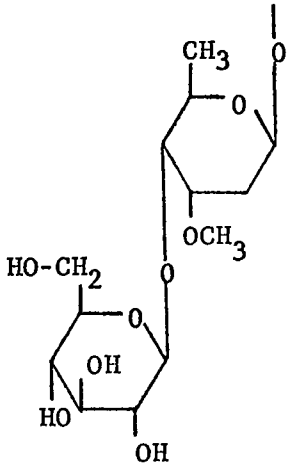
Oleandrigenin: R = OH



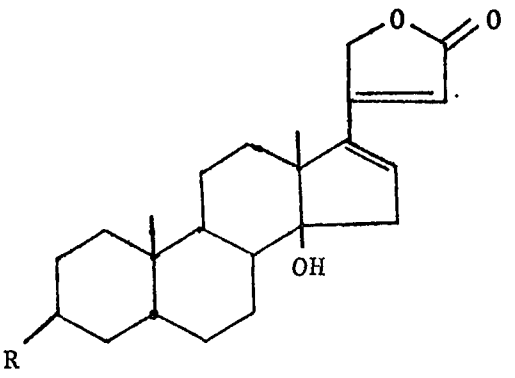
Honghеloside A: R = D-cymarose



Honghеloside C: R =

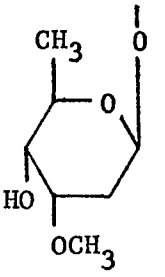


16-Anhydro-oleandrigenin: R = OH



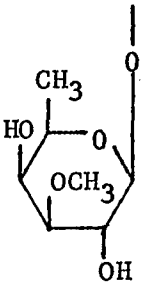
16-Desacetyl-anhydrohongheloside A:

R = D-cymarose



16-Anhydrostrospeside:

R = D-digitalose



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