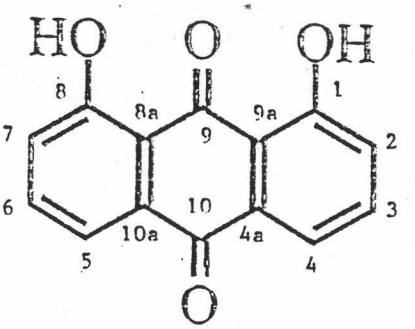
CHAPTER IV

DISCUSSION

The present work had led to the isolation of three anthraquinone aglycones, two phytosterols and two new isocoumarins from the flowering heads of *Xyris indica* Linn. The characterization of the isolated compounds, were achieved by the analyses of ultra violet, infrared, NMR and mass spectra. The structure elucidation of seven isolated compounds are described as follows.

Compound XI-1 was obtained as dark orange plate. The UV absorption spectrum (Figure 16, p.120) exhibited the maxima absorption at 223, 250, 270, 280 and 426 nm. The IR spectrum (Figure 17, p.121) showed OH-stretching at υ_{max} 3453 cm^{-1}, the carbonyl stretching at υ_{max} 1675 cm^{-1} and 1626 cm^{-1} and aromatic vibration at υ_{max} 1600 cm^{-1} and 1568 cm^{-1}. The EI mass spectrum (Figure 21, p.125) showed the molecular ion peak at *m/z* 240 which corresponding to the molecular formula $C_{14}H_8O_4$. The chemical structure of XI-1 is shown below.



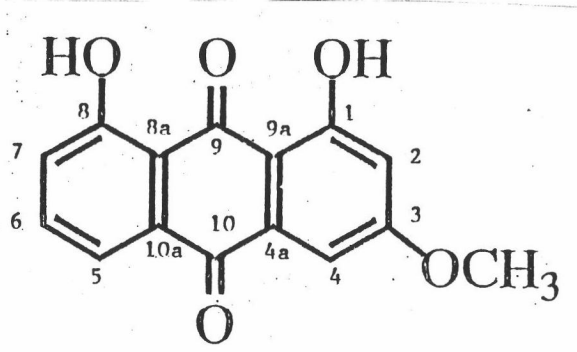
chrysazin

^{1}H-NMR spectral data (Figure 18-19, p.122-123) indicated the presence of two hydroxyl protons appeared as broad singlet at δ 11.98 ppm (H-1 and H-8) downfield due to hydrogen bonding. Two hydroxyl protons occurred at the same chemical shift due to symmetry of structure. The downfield chemical shift of aromatic methine protons were observed at δ 7.23 ppm (dd, J = 1.3, 7.8 Hz, H-2 and H-7), δ 7.62 ppm (dd, J = 1.3, 7.8 Hz, H-3 and H-6) and δ 7.75 ppm (dd, J = 1.3, 7.8 Hz, H-4 and H-5). The equivalent coupling constant (J = 7.8, 8.1 Hz) revealed that H-3 coupled to H-2 and H-4 ; H-6 couple to H-5 and H-7. The chemical shift of H-2 and H-7 showed more upfield than H-3, H-4 and H-6, H-5 because of resonance effect of hydroxy group at position 1 and 8, along with H-7 which also more upfield than H-6 and H-5.

^{13}C-NMR spectrum (Figure 20, p.124) revealed ketone group at δ 181.64 ppm and 193.03 ppm. The hydroxy substituted carbon exhibited signal at δ 162.5 ppm. To confirmed the chemical structure of XI-1, ^{1}H-NMR (in $CDCl_3$) spectral data was compared with the reported from literature (in C_6D_6) (Fournier *et al*, 1975). The pattern in mass spectrum showed peaks at *m/z* 240, 212 and 184 which agreed well with the proposed fragmentation pattern as showed in figure 69, p.92. Thus XI-1 is chrysazin unambiguously.

Compound XI-2 was obtained as brilliant yellow needle. The UV absorption spectrum (Figure 22, p.126) revealed the maxima absorption at 222, 245, 263, 280 and 428 cm^{-1}. The IR spectrum (Figure 23, p.127) showed OH-stretching at υ_{max} 3442 cm^{-1}, the carbonyl stretching at

υ max 1675 cm^{-1} and 1627 cm^{-1} and aromatic vibration at 1609-1560 cm^{-1} The XI-2 was exhibited to have the molecular weight of 270 implying the tentative molecular formula $C_{15}H_{10}O_5$ (Figure 26, p.130). The chemical structure of XI-2 are shown below.



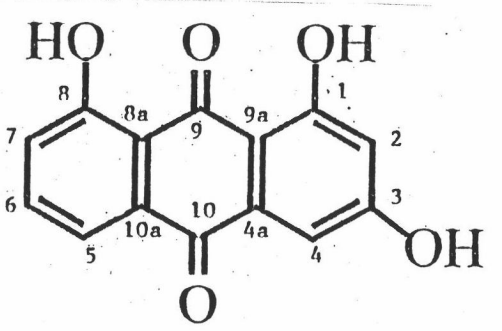
3-methoxy chrysazin

The ^{1}H-NMR spectrum of XI-2 (Figure 24-25, p.128-129) revealed the presence of hydroxy protons at δ 12.11 and 12.19 ppm as broad singlets. The downfield chemical shift of aromatic methine proton were observed at δ 6.61 ppm (d, J = 2.5 Hz, H-2), δ 7.21 ppm (dd, J = 7.6, 1.2 Hz, H-7), δ 7.30 ppm (d, J = 2.5 Hz, H-4), δ 7.56 ppm (dd, J = 7.3, 2.5 Hz, H-6) and = 7.73 ppm (dd, J = 7.3, 2.5 Hz, H-5).

Two doublets at δ 6.61 ppm and 7.30 ppm with a small coupling constant, J = 2.5 Hz, suggested two aromatic protons with a *meta* relationship. The methoxy group was recognized as a singlet at δ 3.87 ppm which expected to substitute at position 3.

Confirming the chemical structure of XI-2, ^{1}H-NMR (in $CDCl_3$) spectral data was compared with published value (in C_6 D_6) (Fournier *et al*, 1975). The proposed fragmentation patterns was shown in figure 69, p.92. Thus, it was concluded to be 3-methoxy chrysazin.

Compound XI-7 was obtained as orange cluster. The UV absorption spectrum (Figure 59, p.163) showed the maxima absorption at 218 (29119), 245 (16433), 266 (16512) and 284 (18534) nm. The IR spectrum (Figure 60, p.164) showed OH-stretching at υ_{max} 3385 cm^{-1} and the carbonyl stretching at υ_{max} 1675 cm^{-1} and 1621 cm^{-1}. The band at 1578 cm^{-1} and 1477 cm^{-1} indicated the presence of aromatic vibration. The EI mass spectrum (Figure 67, p.171) exhibited the molecular ion at *m/z* 256 which corresponding to the tentative molecular formula of $C_{14}H_8O_5$. The chemical structure was shown below.



1,3,8-trihydroxy-9,10-anthraquinone

Preliminary examination of the spectrum in comparison with published proton NMR spectral data for compounds in this series indicated that XI-7 was a chrysazin derivative. The ^{1}H-NMR spectrum of XI-7 (Figure 61-62, p.165-166) revealed the presence of two hydroxyl protons appeared as two singlets at δ 12.03 ppm and δ 12.07 ppm. The downfield chemical shift of five aromatic methine protons were observed at δ 7.33 ppm (dd, *J* = 8.5 Hz,1.2 Hz,H-7), δ 7.74 ppm (dd,*J* = 8.5 Hz, 1.2 Hz, H-6), δ 7.65 ppm (dd, *J* = 8.5 Hz, 1.2 Hz, H-5), δ 7.11 ppm (d, *J* = 2.5 Hz, H-4) and δ 6.58 ppm (d, *J* = 2.5 Hz, H-2). The equivalent coupling constant indicated that H-6 coupled to H-5 and

H-7. Two sets of doublets centered at δ 6.58 ppm (d, J = 2.5 Hz) and δ 7.11 ppm (d, J = 2.5 Hz) represented the *meta* aromatic protons at the position 2 and 4 of ring C. This also indicated that the remaining aromatic proton at position 3 was substituted with hydroxyl group which corresponded to HREI mass spectrum (Figure 67, p.171). The moisture of DMSO-d_6 as solvent caused the disappearing of 3-hydroxyl proton. Confirmation by ^{13}C-NMR (Figure 63, p.167) which provided 14 lines of carbon signals, also indicated that XI-7 has hydroxyl substitute. From DEPT spectrum (Figure 64, p.168), it was shown that XI-7 consisted of 9 quaternary carbons (δ 109.07 ppm,115.60 ppm, 133.12 ppm, 135.13 ppm, 161.24 ppm, 164.53 ppm, 165.76 ppm, 181.29 ppm and 190.07 ppm) and 5 methine carbons (δ 107.94 ppm, 108.84 ppm, 119.29 ppm, 124.47 ppm and 136.76 ppm).

Compound XI-7 was first reported from the symbiosis of bacterium and insectpathogen nematode (Sztaricskai *et al*, 1992). The ^{1}H and ^{13}C-NMR spectral data of those report previously were ambiguously assigned. In this work, ^{13}C-^{1}H and ^{13}C-^{1}H long range COSY were undertaken to determined the completely assignment of XI-7. Confirmation by HMBC spectrum (Figure 65-66, p.169-170), the significant long range ^{1}H-^{13}C correlations contour were indicated by arrows in figure 68, p.92. The assignment of carbon and proton signals were summerized in Table 2. The HREIMS spectrum showed the characteristic fragmentation which confirmed the structure of XI-7 as shown in figure 69,p.92. Thus,it was concluded to be 1,3,8-trihydroxy-9,10-anthracenedione. This is the first reported of 3-hydroxy chrysazin occurring in higher plants.

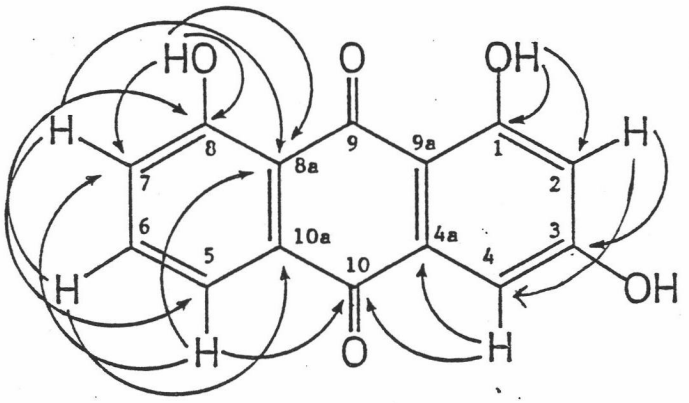
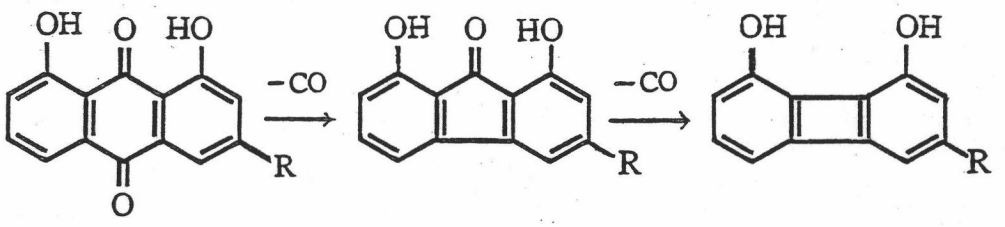


Figure 68 The C-H correlations observed from HMBC spectrum of XI-7



		MW	*m/z*	*m/z*
XI-1	R = H	240	212	184
XI-2	R = OCH_3	270	242	214
XI-7	R = OH	256	228	200

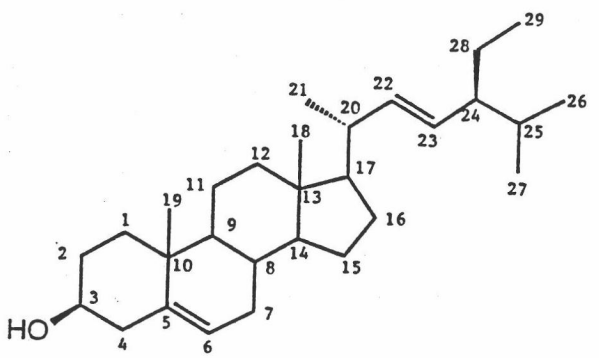
Figure 69 The proposed fragmentation patterns of XI-1, XI-2 and XI-7 from EI mass spectrum.

Table 2 Carbon and proton assignments of XI-7 and long-range correlation between carbon and proton by HMBC spectrum.

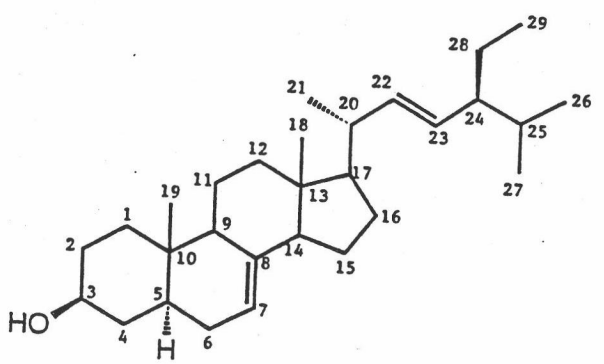
Position	δ C (ppm)	δ H (ppm) (multiplicity, *J* Hz)	^{13}C-^{1}H long-range correlation observed in HMBC spectrum
1	164.53	12.03 (s)	H-1
2	107.94	6.58 (d, 2.5)	H-1
3	165.76	-	H-2
4	108.84	7.11 (d, 2.5)	H-2
4a	109.07	-	H-4
5	119.29	7.65 (dd,8.5,1.2)	H-7
6	136.76	7.74 (dd,8.5,1.2)	-
7	124.47	7.33 (dd,8.5,1.2)	H-8, H-5
8	161.24	12.07 (s)	H-8, H-6
8a	115.60	-	H-8, H-5, H-7
9	190.07	-	-
9a	135.13	-	-
10	181.29	-	H-4, H-5
10a	133.12	-	H-6

Two phytosterols, compound XI-4 and XI-5 were obtained as white needles. They gave violet colour with Leibermann Burchard test. The spots of XI-4 and XI-5 could not detected on the silica gel 60 F 254 plate under UV light at 254-365 nm. This suggested that these compounds have less unsaturated dienes. Their melting points at 169 °c and 170 °c were also similar to the melting point of stigmasterol and α -spina sterol respectively (Devon and Scott, 1972).

The molecular formula of XI-4 and XI-5 were deduced to be $C_{29}H_{48}O$ from their molecular ion at *m/z* 412 in EIMS (Figure 42, p.146 and Figure 48, p.152). The chemical structures of XI-4 and XI-5 are shown below.



stigmasterol



α -spinasterol

Compound XI-4 had IR absorption spectrum (Figure 37, p.141) which exhibited O-H stretching at 3433 cm^{-1}, C-O stretching at 1060 cm^{-1}, C-H stretching of methyl and methylene group at 2936 and 2867 cm^{-1}, C-H bending of methyl and methylene group at 1381 and 1462 cm^{-1}.

The ^{1}H-NMR spectrum of compound XI-4 (Figure 38-39, p.142-143) revealed the signals at δ 0.65 - 1.01 ppm corresponding to the signals of methyl proton substituted at C-18, C-19 and at side chain of steroidal compound. The signal at δ 1.1 - 2.3 ppm were the signal of methylene and methine protons of steroids. The signal at δ 3.5 ppm (m) was the signal of oxygenated proton at C-3. The olefinic signal at δ 5.14 ppm (dd, J = 11.9, 8.5) and 4.98 ppm (dd, J = 11.9, 8.5) were *trans*-disubstituted vinyl proton (H-22 and H-23). The olefinic signal at δ 5.33 ppm (br d) could be assigned as H-6 which was trisubstituted vinyl proton.

The ^{13}C-NMR spectrum assignment could be made as shown in figure 40-41,p.144-145.The signals of methine carbons were at δ 121.69 ppm, 129.24 ppm, 138.30 ppm and 140.73 ppm. The signal at δ 71.77 ppm was the signal of carbon that attach to oxygen at C-3.

The assignment for each proton and ^{13}C-NMR spectrum of XI-4 established in this work clearly supported by the previous suggestion (Wright *et al*, 1978).

The pattern in mass spectrum data showed peak at *m/z* 412, 397, 394,379, 253 and 211 which agreed well with the proposed fragmentation pattern as shown in figure 70.

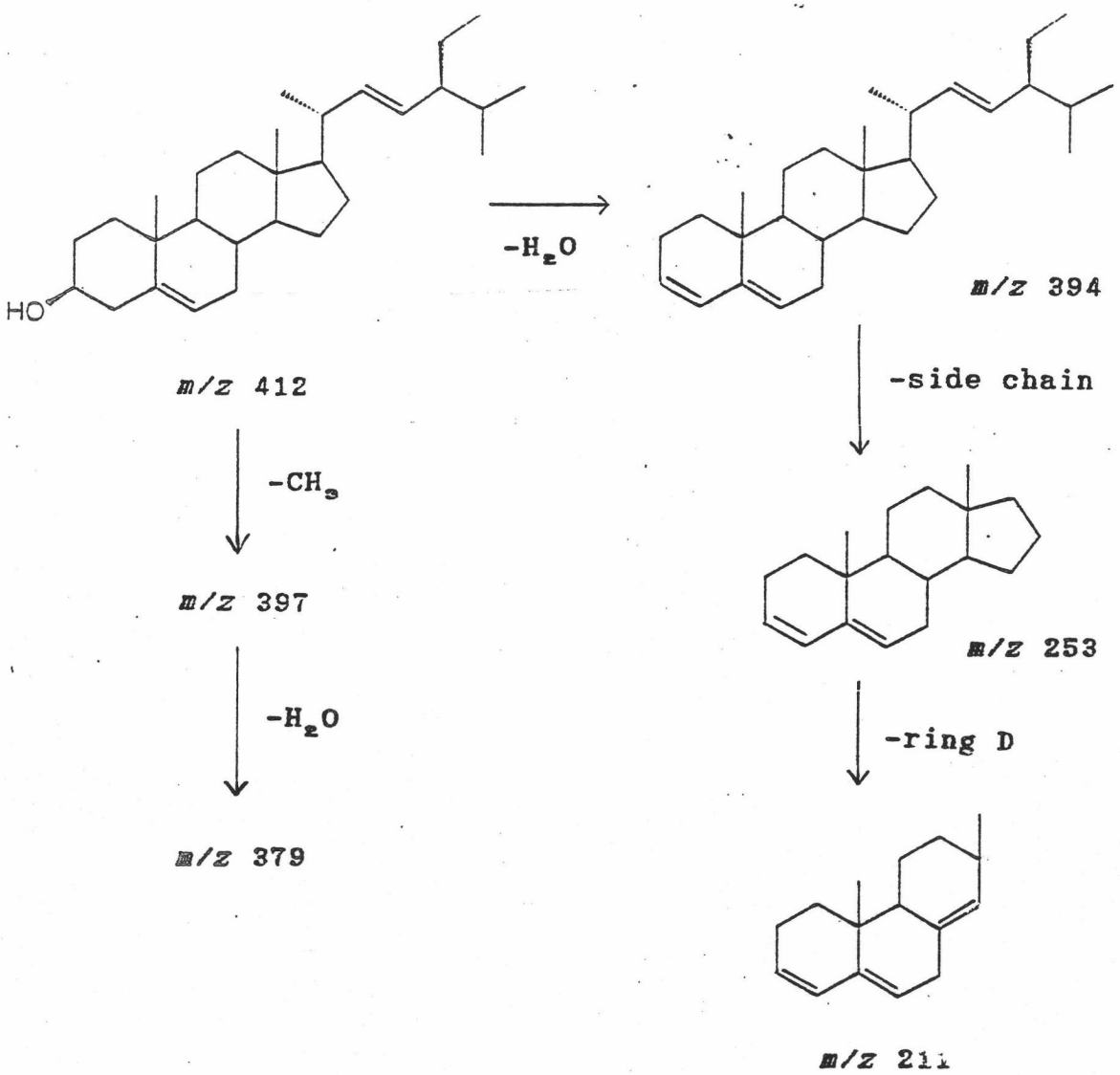


Figure 70 The proposed fragmentation patterns of XI-4

The chemical test and spectroscopic data indicated that XI-4 is a steroid compound, namely stigmasterol.

The IR spectrum of compound XI-5 (Figure 43, p.147) revealed O-H stretching at 3436 cm^{-1}, C-O stretching at 1041 cm^{-1}, C-H stretching of methyl and methylene groups at 2957 and 2870 cm^{-1}, C-H bending of methyl and methylene groups at 1382 and 1447 cm^{-1} and C-H bending of aromatic group at 971 cm^{-1}.

The ^{1}H-NMR spectrum (Figure 44-45, p.148-149) exhibited the signals of 0.51 - 2.2 ppm which was the signals of methyl protons that are substituted at C-18, C-19 and at side chain of steroidal compound and the signal of methylene and methine protons. The signal at δ 3.5 ppm is the oxygenated proton at C-3. The olefinic signal at δ 5.13 ppm (dd, J = 15.15, 8.5) and δ 5.01 ppm (dd, J = 15.25, 8.5) were *trans*-disubstitute vinyl proton (H-22 and H-23). The olefinic signal at δ 5.13 ppm (br d) could be assigned as H-6 which was trisubstituted vinyl proton.

The ^{13}C-NMR spectrum assignment could be made as shown in figure 46-47, p.150-151. The signals of methine carbons were at δ 117.46 ppm, 129.47 ppm, 138.14 ppm and 139.55 ppm. The signal at δ 71.05 ppm was the signal of carbon that attach to oxygen at C-3.

The pattern in mass spectrum data exhibited peak at m/z 412, 397 and 271 which agreed well with the proposed fragmentation pattern as shown in figure 71, p.98.

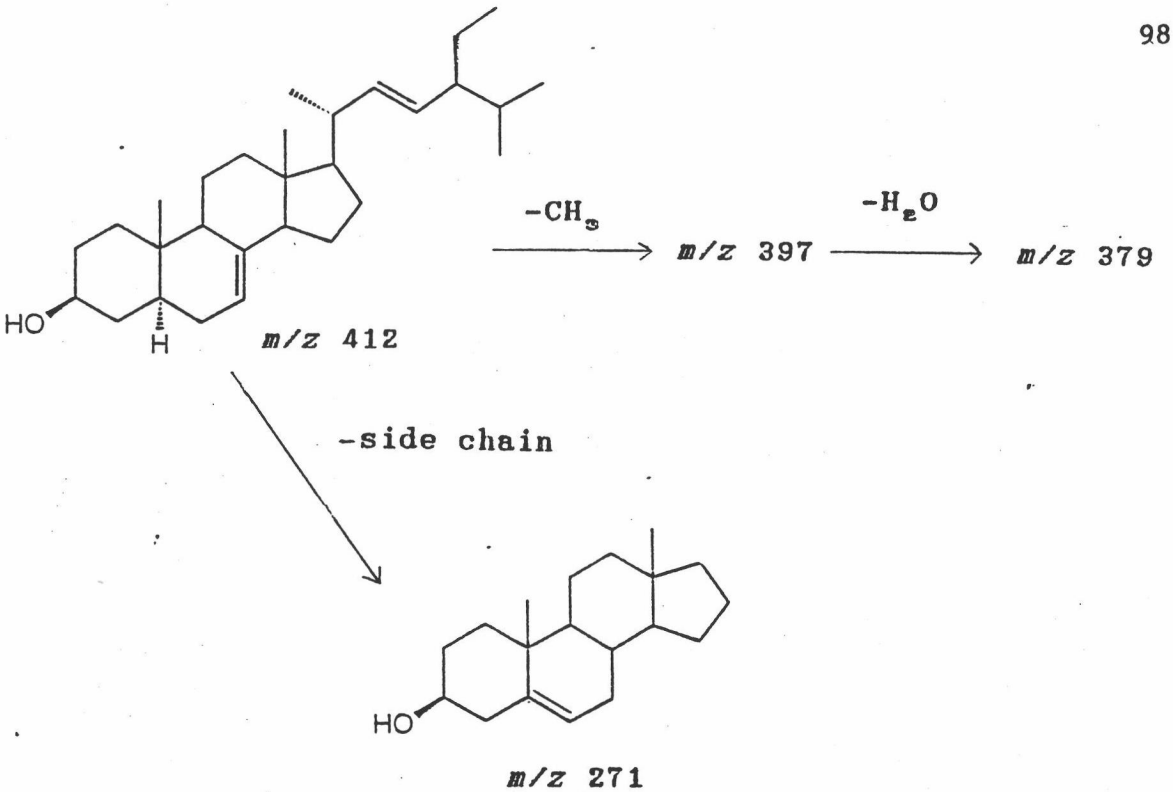
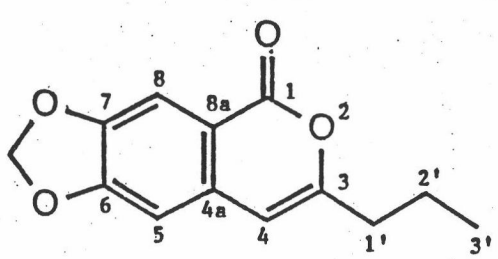


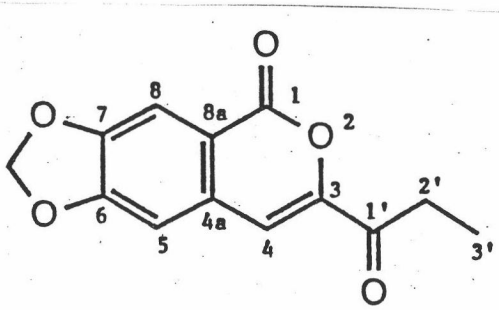
Figure 71 The proposed fragmentation patterns of XI-5

The chemical test and spectroscopic data indicated XI-5 is α -spinasterol which supported by the previous suggestion (Akihisa *et al*, 1986).

Purification and extraction of the flowering heads of *Xyris indica* Linn. also resulted in the isolation of two new isocoumarin (XI-3 and XI-6) as shown below.



xyridin A



xyridin B

Compound XI-3, m.p. 67 - 68 °c, was obtained as a pale yellow plate. The molecular formula of XI-3 was deduced to be $C_{13}H_{12}O_4$ (Calcd. 232.0732 amu) from its molecular ion at *m/z* 232.0731 in the HREIMS (Figure 36, p.140). The benzopyrone structure was evident from its UV absorptions at 334 (4478) and 282 (5892) nm (Figure 27, p.131), and the pyrone-carbonyl stretching frequency was found in the region 1725 cm^{-1} from IR spectrum (Murray *et al*, 1982) (Figure 28, p.132). The presence of a methylenedioxy group at C-6 and C-7 was indicated by the isolated methylene proton signal at δ 6.03 ppm (s). The ^{1}H-NMR spectrum (Figure 29-30, p.133-134) exhibited aromatic proton resonance of H-5 at δ 6.66 ppm(s), H-8 at δ 7.51 ppm(s) and H-4 at δ 6.09 ppm(s).

Analysis of the ^{13}C-NMR (Figure 31, p.135) and DEPT spectra of XI-3 (Figure 32, p.136) revealed the presence of 3 methylene carbons (δ 20.18 ppm, 35.16 ppm and 102.02 ppm), 3 aromatic metheine carbons (δ 102.96 ppm, 103.50 ppm and 107.19 ppm), one methyl carbon (δ 13.40 ppm), and 6 quaternary carbons (δ 114.48 ppm, 135.19 ppm, 147.72 ppm, 153.51 ppm, 156.99 ppm and 162.63 ppm) including one carbonyl carbon. The n-propyl side chain could be assigned from ^{1}H-signals at δ 0.93 ppm, 1.66 ppm and 2.42 ppm [t (J = 7.3 Hz), tq (J = 7.3, 7.3 Hz) and t (J = 7.3 Hz)]. It could be interpreted from the above data that there are a number of alternatives structure of XI-3 to be deduced : (a) a 3-n propyl or 4-n-propyl-6,7-(methylenedioxy)-1H-2-benzopyran-1-one and (b) a 3-n-propyl- or 4-n-propyl-6,7-(methylenedioxy)-2H-1-benzopynan-2-one. In order to evaluate the structure more explicitly,a detailed examination of the C-H COSY spectrum (Figure 33, p.137) and the HMBC spectrum

(Figure 34-35, p.138-139) were undertaken. The significant long range ^{1}H-^{13}C correlations contour were indicated by arrows in figure 72. The assignments of carbon and proton signals of XI-3 were summerized In table 3. The quaternary carbon at δ 156.99 ppm (C-3) was correlated with the methylene proton at δ 2.42 ppm (H-1′) and δ 1.66 ppm (H-2′) and with the olefinic proton at δ 6.09 ppm (H-4). The carbonyl carbon at δ 162.63 ppm (C-1) was correlated with the olefinic proton at δ 7.51 ppm (H-8). The quaternary carbon at δ 144.48 ppm (C-4a) was correlated with the olefinic protons at δ 6.09 ppm (H-4) and 6.66 ppm (H-5). The quaternary carbon at δ 135.19 ppm (C-8a) was correlated with the olefinic proton at δ 7.51 (H-8).

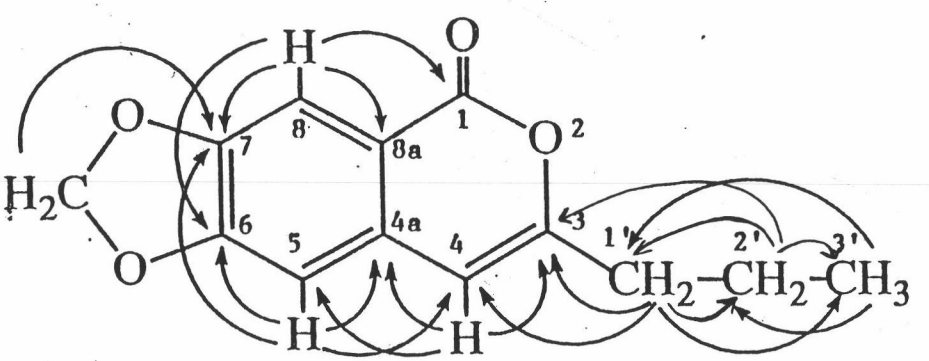


Figure 72 The C-H correlations observed from HMBC spectrum of XI-3

The HREIMS spectrum of XI-3 showed the characteristic fragmentation which confirmed the structure of XI-3. The patterns of fragmentation were shown in figure 73, p.101. These results strongly support the proposed structure of XI-3 as 3-n-propyl-6,7-(methylenedioxy)-1H-2-benzopyran-1-one, a new isocoumarin named xyridin A.

Table 3 Carbon and proton assignments of XI-3 and long-range correlation between carbon and proton by HMBC spectrum.

Position	δ C (ppm)	δ H (ppm) (multiplicity, *J* Hz)	^{13}C-^{1}H long-range correlation observed in HMBC spectrum
1	162.63	-	H-8
3	156.99	-	H-4, H-1′, H-2′
4	102.96	6.09 (s)	H-5, H-1′
4a	114.48	-	H-4, H-5
5	103.50	6.66 (s)	H-4
6	147.72	-	H-5, H-8
7	153.51	-	H-5, H-8, H-CH_2
8	107.19	7.51 (s)	-
8a	135.19	-	H-8
-O-CH_2-O-	102.02	6.03 (s)	-
1′	35.16	2.42 (t, 7.3)	H-2′, H-3′
2′	20.18	1.66 (tq, 7.3,7.3)	H-3′, H-1′
3′	13.40	0.93 (t, 7.3)	H-1′, H-2′

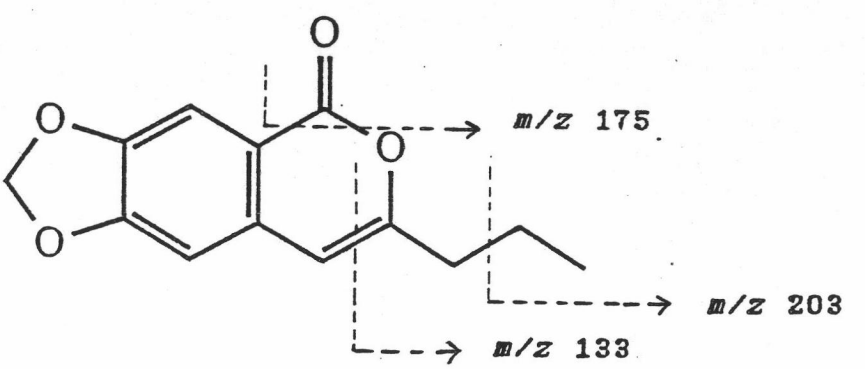


Figure 73 The proposed fragmentation patterns of XI-3

Compound XI-6, m.p. 198 - 199 °c, was obtained as a colourless plate, possessing the molecular formula of $C_{13}H_{10}O_5$ as determined by HREIMS spectrum (Figure 58, p.162). A variety of spectroscopic techniques were employed to determine the structure of XI-6 and indicated a close structural relationship with XI-3 as both compounds exhibited the same carbon framework. UV spectrum of XI-6 showing absorption maxima at 333 (14293) and 262 (36808) nm, suggested the feature of benzopyrone (Figure 49, p.153). The two carbonyl stretching frequency were found in the region of 1695 and 1733 cm^{-1}, in IR spectrum (Figure 50, p.154). ^{1}H-NMR spectrum of XI-6 (Figure 51-52, p.155-156) indicated the presence of three aromatic protons at δ 7.24 (s) ppm, 6.92 ppm (s) and 7.62 ppm (s) and two methylene protons at δ 6.11 ppm (s) and 2.92 ppm (q), suggested a close resemblance to XI-3 based on the 6,7-(methylenedioxy)-1H-2-benzopyran-1-one skeleton. Further more, the triplet at δ 1.14 ppm and quartet at δ 2.92 ppm (J = 7.3 Hz) indicated the CH_3-CH_2- side chain.

In the ^{13}C-NMR and DEPT spectra (Figure 53-54, p.157-158), it was shown that XI-6 consisted of 2 methylene carbons (δ 31.63 ppm and 102.71 ppm), 3 methine carbon (δ 106.32 ppm, 108.09 ppm and 108.52 ppm), one methyl carbon (δ 7.33 ppm) and 7 quaternary carbons (δ 118.39 ppm, 132.57 ppm, 148.68 ppm, 150.34 ppm, 153.86 ppm, 160.42 ppm and 194.91 ppm) (including two carbonyl carbons). It is clearly shown that XI-6 has an additional carbonyl on the propyl side chain indicated by the presence of oxopropyl group from the NMR and MS data, confirmation by C-H COSY (Figure 55, p.159) and C-H long range connectivity of XI-6 by HMBC spectrum (Figure 56-57, p.160-161). The significant long range ^{1}H-^{13}C correlations contour were indicated

by arrows in figure 74, p.103. The assignments of carbon and proton signals were summerized in Table 4. It indicated that the carbonyl carbon (C-1′) at δ 194.91 ppm of oxopropyl side chain was correlated with the olefinic proton at δ 7.24 ppm (H-4), methylene proton at δ 2.92 ppm (H-2′) and methyl proton at δ 1.14 ppm (H-3′). The quaternary carbon at δ 148.68 (C-3) was correlated with the olefinic proton at δ 7.24 ppm (H-4). The carbonyl carbon at δ 160.42 ppm (C-1) was correlated with the olefinic proton at δ 7.62 ppm (H-8). The quaternary carbon at δ 118.39 ppm (C-4a) was correlated with the olefinic protons at δ 7.24 ppm (H-4) and δ 6.92 ppm (H-5). The quaternary carbon at 132.57 ppm (C-8a) was correlated with olefinic proton at δ 7.62 ppm (H-8).

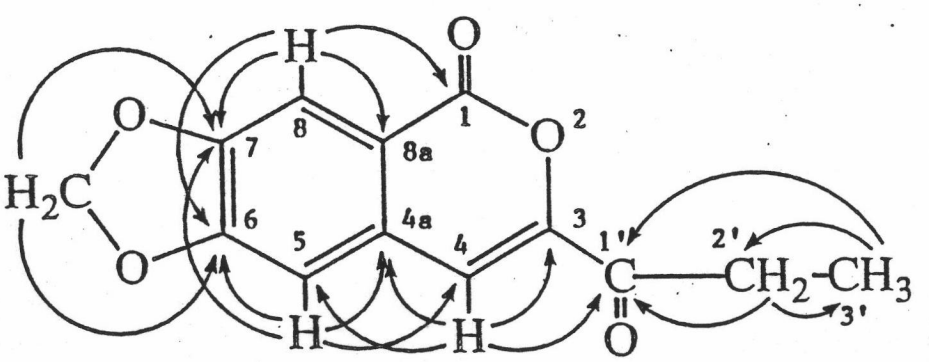


Figure 74 The C-H correlations observed from HMBC spectrum of XI-6

The HREIMS spectrum of XI-6 showed the characteristic fragmentation which confirmed the structure of XI-6. The patterns of fragmentation were shown in figure 75, p.104. The structure of XI-6 was thus determined to be 3-(1′-oxopropyl)-6,7-(methylenedioxy)-1H-2-benzopyran-1-one unambiguously and named xyridin B.

Table 4 Carbon and proton assignments of XI-6 and long-range correlation between carbon and proton by HMBC spectrum.

Position	δ C (ppm)	δ H (ppm) (multiplicity, *J* Hz)	^{13}C-^{1}H long-range correlation observed in HMBC spectrum
1	160.42	-	H-8
3	148.68	-	H-4
4	108.52	7.24 (s)	H-5
4a	118.39	-	H-4, H-5
5	106.32	6.92 (s)	H-4
6	150.34	-	H-5, H-CH_2, H-8
7	153.86	-	H-5, H-CH_2, H-8
8	108.09	7.62 (s)	-
8a	132.57	-	H-8
-O-CH_2-O-	102.71	6.11 (s)	-
1′	194.91	-	H-4, H-2′, H-3′
2′	31.63	2.92 (q, 7.3)	H-3′
3′	7.33	1.14 (t, 7.3)	H-2′

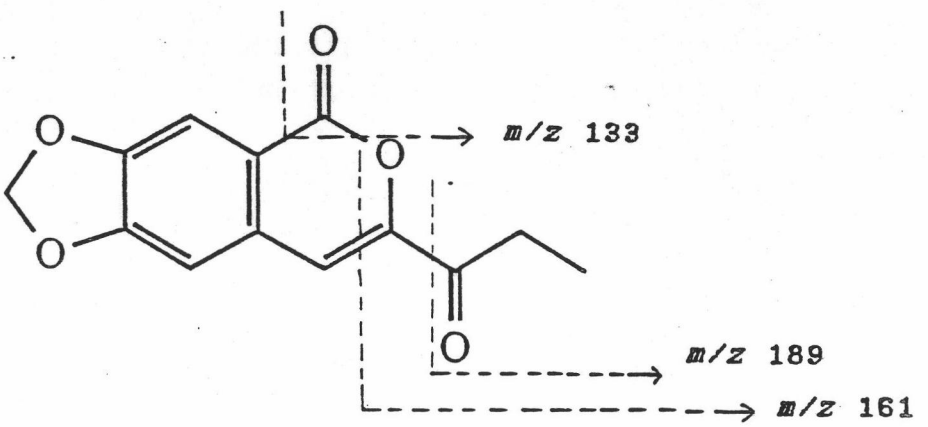


Figure 75 The proposed fragmentation patterns of XI-6

From a biogenetic point of view, there seems to be no doubt that xyridin A (XI-3) and xyridin B (XI-6) arise from the acetate-malonate pathway through cyclization reactions of a C_{12} polyketide chain. This secondary metabolic route appears to be responsible for the other constituents which have so far been isolated from *Xyris semifuscata* (Fournier *et at*, 1975).

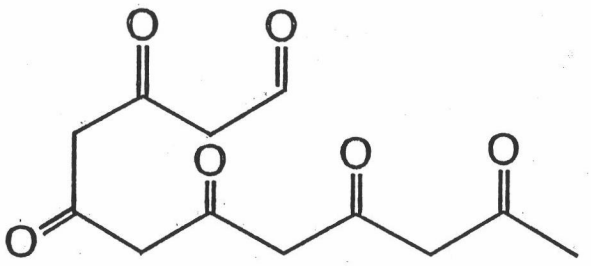


Figure 76 Hexaketide for the formation of xyridins skeleton.

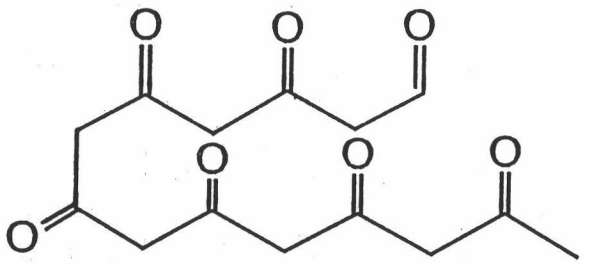


Figure 77 Heptaketide for the formation of xyris anthraquinone skeleton.

It is interested to note that both isocoumarins and anthraquinones in *Xyris indica* are derived from acetate-malonate pathway. Xyridin A and B are composed of 6 acetate units (Figure 76) whilst the backbone skeleton of the xyris anthraquinones required 7 acetate units (Figure 77).