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NEW EUDESMANOLIDES FROM *SPHAERANTHUS INDICUS*

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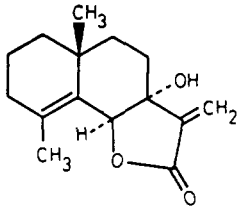
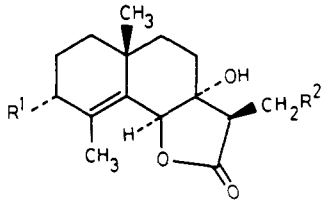
ABSTRACT.—Three new eudesmanolides, 11α,13-dihydro-3α,7α-dihydroxyfrullanolide [**3**], 11α,13-dihydro-7α,13-dihydroxyfrullanolide [**4**], and 11α,13-dihydro-7α-hydroxy-13-methoxyfrullanolide [**5**], were isolated from the flowers of *Sphaeranthus indicus*. Their structures were determined by 2D nmr and other spectroscopic techniques.

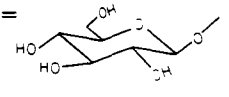
Sphaeranthus indicus L. (Compositae) has long been used in indigenous medicine in the treatment of styptic gastric disorders. The paste prepared from roots and the aerial parts is useful in skin diseases. The powder of seeds, flowers, and roots is anthelmintic and is used for the treatment of glandular swellings, bronchitis, jaundice, and nervous depressions (1–5).

In earlier studies we described the isolation of an antimicrobial compound, 7-hydroxyfrullanolide [**1**] (6), and an immune-stimulating glycoside **2** (7), which to our knowledge are the first examples of 7-hydroxylated eudesmanolides. The aglycone **3** was also obtained via enzymatic degradation of the latter (7). We describe here the isolation of **4** and **5**, as well as that of **3** from the same plant. [While this paper was in preparation, the isolation of **3** from a different species of the Compositae appeared (8)].



1



2 R^1=  , R^2=H

3 R^1=OH, R^2=H
4 R^1=H, R^2=OH
5 R^1=H, R^2=OMe

All three compounds **3**, **4**, and **5** showed only end absorption in their uv spectra. The ir spectra of all the compounds showed characteristic 5-membered lactone absorptions (1752, 1745, and 1757 cm^{-1}, respectively), and revealed the presence of OH and non-conjugated olefin functions.

The overall mass spectral pattern of the three compounds **3**, **4**, and **5** in comparison to that of **1** indicated that they were all eudesmanolides. Their molecular ions were confirmed by fabms and fdms. The mass spectrum of **3** showed a molecular ion at *m/z* 266.1522 ($C_{15}H_{22}O_4$, calcd 266.1518). The other prominent peaks in the eims were at *m/z* 251.1297 ($C_{14}H_{19}O_4$), 248.1427 ($C_{15}H_{20}O_3$), 233.1192 ($C_{14}H_{17}O_3$), 215.1084 ($C_{14}H_{15}O_2$), and 187.1131 ($C_{13}H_{15}O$). The ions at *m/z* 251 and 248 are generated by the loss of a methyl group and an H_2O molecule from the parent ion, respectively. The ions at *m/z* 233 and 215 correspond to successive losses of H_2O molecules from the ion at 251. These losses indicated the presence of at least two OH groups in the molecule. The peak at *m/z* 187 is generated by the loss of CO from the ion at *m/z* 215. The eims of compound **4** showed the molecular ion at *m/z* 266.1522

($C_{15}H_{22}O_4$, calcd 266.1518). Other prominent peaks were at *m/z* 251.1286 ($C_{14}H_{19}O_4$), 233.1185 ($C_{14}H_{17}O_3$), 215.1079 ($C_{14}H_{15}O_2$), and 161.0968 ($C_{11}H_{13}O$). The ions at *m/z* 251, 233, and 215 are generated by loss of a methyl group, followed by successive losses of two H_2O molecules from the molecular ion. Thus this compound also contains at least two hydroxy functions. The ion at *m/z* 161 corresponds to the loss of a $C_3H_2O_2$ unit and a molecule of hydrogen from the ion at *m/z* 233. Compound **5** showed the molecular ion at *m/z* 280.1689 ($C_{16}H_{24}O_4$, calcd 280.1684). Other significant peaks were at *m/z* 265.1462 ($C_{15}H_{21}O_4$), 247.1344 ($C_{15}H_{19}O_3$), 233.1197 ($C_{14}H_{17}O_3$), 215.1091 ($C_{14}H_{15}O_2$), 187.1154 ($C_{13}H_{15}O$), and 161.6989 ($C_{11}H_{13}O$, 100%). The ions at *m/z* 265 and 233 are generated by successive losses of a methyl group and a molecule of MeOH from the parent ion, while the one at *m/z* 247 is generated by the loss of a molecule of H_2O from the ion at *m/z* 265. The subsequent peaks are generated from the peak at *m/z* 233 in the same manner as described above.

The ^{13}C-nmr spectra (Table 1) of compounds **3** and **4** showed 15 carbon atoms in each skeleton, while compound **5** has 16 carbon atoms in its molecule. Multiplicities of carbon signals were determined by the DEPT pulse sequence (9,10). Compound **3** has three CH, four CH_2, three Me, and (by difference) five quaternary carbon atoms, while compound **4** has two CH, six CH_2, three Me, and five quaternary carbons. Similarly compound **5** has two CH, six CH_2, three Me, and five quaternary carbons. The sixteenth carbon in this compound is due to the presence of a methoxy group.

TABLE 1. ^{13}C-nmr Chemical Shifts (ppm) of Compounds **3**, **4**, and **5**.

Carbon	Compound		
	3	**4**	**5**
C-1	33.8	39.3	39.3
C-2	24.9	18.1	18.1
C-3	69.8	33.4	33.2
C-4	139.0	140.7	140.5
C-5	131.3	126.3	126.5
C-6	80.1	80.7	79.8
C-7	77.3	76.9	77.9
C-8	33.7	25.1	25.2
C-9	33.8	33.4	34.1
C-10	34.8	33.0	33.1
C-11	47.7	54.7	52.7
C-12	176.6	175.4	173.3
C-13	7.8	57.6	68.4
C-14	18.0	19.3	19.4
C-15	24.2	25.4	25.4
OMe	—	—	59.4

The ^{1}H-nmr spectra (Table 2) of compounds **3** and **4** indicated the presence of twenty-two hydrogen atoms, while compound **5** contains twenty-four hydrogen atoms in the molecule. The protons were assigned on the basis of a number of 2D nmr studies (see below). The angular methyl group of compounds **3** and **4** resonates at δ 1.00, while that of **5** resonates at δ 1.03. The vinylic methyl group resonates at δ 1.92, 1.71, and 1.78, respectively. The signal for H-6 in each compound appears as a doublet showing long range coupling with H-8 ($J_{6,8}$ = 1.2, 1.3, and 1.3 Hz, respectively), and resonates at δ 4.99, 5.00, and 5.01, respectively. In compound **3** a broad doublet of 1H resonating at δ 3.99 (J = 2.0, $W_{1/2}$ = 10 Hz) was assigned to H-3. A 1H quartet at δ 2.75 (J = 7.2 Hz), and a 3H doublet at δ 1.15 (J = 7.2 Hz) were assigned to H-11 and the 13-methyl group, respectively. In the case of the compound **4** the protons on C-13 appeared at δ 3.90 (1H, dd, J_{gem} = 11.3, J_{vic} = 7.4 Hz) and δ 4.05 (1H, dd, J_{gem} = 11.3, J_{vic} = 6.4 Hz). The signal for H-11 could be easily recognized as a doublet of a doublet, resonating at δ 3.05 (J = 7.4, 6.4 Hz). Similarly in the case of compound **5**, the two protons on C-13 resonated at δ 3.72 (1H, dd, J_{gem} = 9.8, J_{vic} = 10.7 Hz) and δ 3.85 (1H, dd, J_{gem} = 9.8, J_{vic} = 5.2 Hz). The signal for H-11 appeared at δ 3.15 (1H, dd,

TABLE 2. ^{1}H-nmr Spectra of Compounds **3**, **4**, and **5**, ppm (multiplicity).

Proton	3	4	5
H-1α	1.90 m	1.41 m	1.45 m
H-1β	1.70 m	1.35 m	1.41 m
H-2α	2.10 m	1.70 m	1.91 m
H-2β	2.0 m	1.60 m	1.62 m
H-3α		2.10 m	2.10 m
H-3β	3.99 d-like (J = 2.0, $W_{1/2}$ = 10 Hz)	2.15	2.20 m
H-6α	4.99 d (1.2 Hz)	5.00 d (1.3 Hz)	5.01 d (1.3 Hz)
7-OH	2.35	2.55	2.75
H-8α	1.55 m	1.63 m	1.63 m
H-8β	1.70 m	1.90 m	1.92 m
H-9α	1.60 m	1.55 m	1.35 m
H-9β	1.40 m	1.41 m	1.41 m
H-11α	2.75 q (7.2 Hz)	3.05 dd (7.4, 6.4 Hz)	3.15 dd (10.7, 5.2 Hz)
H-13	1.15 d (7.2 Hz)	3.90 dd (11.3, 7.4 Hz)	3.72 dd (9.8, 10.7 Hz)
H-13′		4.05 dd (11.3, 6.4 Hz)	3.85 dd (9.8, 5.2 Hz)
Me-14	1.92 s	1.71 s	1.78 s
Me-15	1.00 s	1.00 s	1.03 s
OMe	—	—	3.38 s

J = 10.7, 5.2 Hz). This coupling pattern was verified by double resonance experiments. The downfield singlet at δ 3.38 is due to the methoxy group at C-13. The H-H coupling interactions were further confirmed by homonuclear decoupling and COSY-45 experiments. The hetero-COSY spectrum (11) facilitated the assignments of ^{1}H and ^{13}C resonances, while the long range hetero-COSY spectra (12) established correlations between carbon and distant protons.

The NOESY spectra of compounds **3**, **4**, and **5** showed cross-peaks between H-6 and Me-14, as well as between H-6 and H-11. No cross-peaks, however, were observed between H-6 and Me-15 in any of the compounds. This established the α orientation of H-6 and H-11 in all cases. In the case of compound **3**, weak cross-peaks were observed between Me-15 (δ 1.0) and H-3 (δ 3.99), suggesting the α orientation of the OH group attached to C-3. The relatively upfield chemical shift of C-3 (δ 69.8) is also consistent with an axial orientation of the oxygen (13). The NOESY interactions were further confirmed by nOe difference measurements. The stereochemistry at C-7 has been assigned on the basis of biogenetic considerations. Such an assignment in the past has been confirmed through X-ray studies (14).

Compound **3** was identical in all respects with that obtained via enzymatic hydrolysis of the glycoside **2**. In order to investigate the likelihood that **4** or **5** may be artifacts of isolation, compound **1** was allowed to react with anhydrous MeOH as well as with MeOH-H_2O (1:1), both in the presence of *p*-toluenesulfonic acid as well as that of Si gel for extended periods. In neither case could **4** or **5** be detected on tlc. Similarly the reaction of **4** with anhydrous MeOH in the presence of either *p*-toluenesulfonic acid or Si gel does not yield **5**. Although these experiments do not completely rule out the possibility of **4** or **5** being an artifact, they do indicate that both are probably genuine natural compounds.

EXPERIMENTAL

The ^{1}H- and ^{13}C-nmr spectra of **4** and **5** were recorded in $CDCl_3$ at 400 MHz and 100 MHz, respectively, on a Bruker AM-400 nmr spectrometer, while those of compound **3** were recorded at 300 MHz and 75 MHz, respectively on a Bruker AM-300 nmr spectrometer. The 2D spectra were also obtained on the same instrument. The uv and ir spectra were obtained on Shimadzu UV-240 and JASCO A-302 spectrometers, respectively.

The mass spectra were obtained either on MAT-312 or on JEOL JMS-HX110 instruments. Optical rotations were recorded on a JASCO DIP-360 digital polarimeter. Cc was performed over Si gel (Merck, 70–230 mesh). Preparative tlc utilized Si-gel PF_{254} coated to 0.5 mm thickness. The tlc was carried out on Si-gel PF_{254}, precoated plates (Merck).

PLANT MATERIAL.—The plant material (which was of Indian origin) was commercially purchased and identified by Hamdard National Foundation, a leading manufacturer of herbal medicine in the subcontinent. A specimen has been deposited at the Herbarium of the Department of Botany, University of Karachi under voucher No. 33331 (KUH).

ISOLATION OF 11α,13-DIHYDRO-3α,7α-DIHYDROXYFRULLANOLIDE [**3**], 11α,13-DIHYDRO-7α,13-DIHYDROXYFRULLANOIDE [**4**], AND 11α,13-DIHYDRO-7α-HYDROXY-13-METHOXYFRULLANOLIDE [**5**].—The EtOH extract (245 g) of the dried flowers (40 kg) of *S. indicus* was partitioned between H_2O and hexane, and the hexane-soluble material was separated. The aqueous suspension was extracted with Et_2O, and the crude Et_2O extract (16 g) was chromatographed (in 8-g portions) on Si gel columns, using petroleum ether (40–60°)-EtOAc (95:5). The fractions showing similar behavior on tlc [Si gel, CH_2Cl_2-MeOH (92:8)] using vanillin/phosphoric acid (15) visualization were combined. The fractions containing **1** were separated. The impure compounds **3**, **4**, and **5** were further purified by repeated preparative tlc using CH_2Cl_2-MeOH (92:8) to afford pure **3** (35 mg), mp 63°, $[\alpha]^{26}D$ −7.1 (c = 0.112, $CHCl_3$); **4** (80 mg) mp 90.5–91.5°, $[\alpha]^{26}D$ −80.0 (c = 0.2, $CHCl_3$); and **5** (10 mg), oil, $[\alpha]^{26}D$ −18.9 (c = 0.18, $CHCl_3$). For ^{13}C- and ^1H-nmr spectra, see Tables 1 and 2.

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