Journal of Natural Products
Vol. 47, No. 6, pp. 977-982, Nov-Dec 1984

OFFICIGENIN, A NEW SAPOGENIN OF *GUAIACUM OFFICINALE*

VIQAR UDDIN AHMAD,* NASREEN BANO, and SHAHEEN BANO

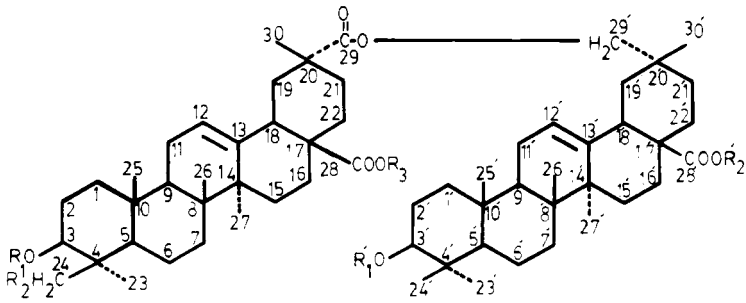
H.E.J. Research Institute of Chemistry, University of Karachi, Karachi-32, Pakistan

ABSTRACT.—A new sapogenin, named officigenin (**1**), has been isolated from the acidic hydrolysate of the *Guaiacum officinale* saponins. The alkaline hydrolysis of **1** furnished two triterpenoids, namely 3β,29-dihydroxyolean-12-en-28-oic acid (mesembryanthemoidigenic acid) (**2**) and 3β,24-dihydroxyolean-12-en-28,29-dioic acid (**3**). Compounds **1** and **3** are new.

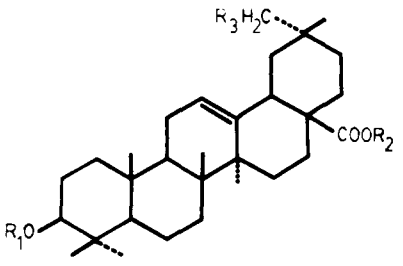
Recently, we reported (1) the isolation and characterization of a new sapogenin, namely 3β,20ξ-dihydroxy-30-norolean-12-en-28-oic acid, and the artifacts 3β-hydroxy-30-norolean-12,19-dien-28-oic acid and its methyl ester together with larreagenin A, β-sitosterol, and oleanolic acid, from the acid hydrolysate of the saponins obtained from the stem bark of *Guaiacum officinale* L. (Zygophyllaceae). We have now succeeded in isolating another sapogenin named officigenin from the same source.

Officigenin (**1**) in the acid hydrolysate was eluted with $CHCl_3$-MeOH (90:10) from the silica gel column (1) and purified through recrystallization from Me_2CO. It analyzed for $C_{60}H_{92}O_9 \cdot H_2O$, and its uv spectrum in MeOH showed only end absorption at 212 nm. The ir spectrum revealed the presence of hydroxyl (3440 cm^{-1}), ester (1728 cm^{-1}), carboxyl (1705 cm^{-1}), and trisubstituted double bond(s) (825 cm^{-1}). On treatment with CH_2N_2, it furnished a dimethyl ester (**1a**), which was acetylated with Ac_2O and pyridine to give a dimethyl ester triacetate (**1b**). The ^{1}H-nmr spectrum of **1a** exhibited singlets due to 11 tertiary methyl groups at δ 0.66, 0.69, 0.75, 0.83, 0.88 (each 3H, s, 5×CH_3), δ 0.96, 1.09, 1.22 (each 6H, s, 6×CH_3); other ^{1}H-nmr signals were an unresolved double doublet centered at δ 2.88 (2H), a multiplet centered at δ 3.25, a singlet at δ 3.61 (6H) due to two COOMe, a singlet at δ 3.69 (2H), and a triplet at δ 5.28 (2H). From the ir and ^{1}H-nmr spectra, **1** appeared to be an ester of two triterpenoids, a carboxylic group of one esterified with an alcohol group of the other. This was also confirmed by alkaline hydrolysis of **1**, which, indeed, afforded a mixture of two triterpenoids. These were separated through silica gel column chromatography yielding 3β,29-dihydroxyolean-12-en-28-oic acid (mesembryanthemoidigenic acid) (**2**) and 3β,24-dihydroxyolean-12-en-28, 29-dioic acid (**3**).

Compound **2** was eluted with C_6H_6-EtOAc (1:1) and crystallized from MeOH as colorless needles. Its mass spectrum exhibited a molecular ion peak at *m/z* 472 corresponding to the formula $C_{30}H_{48}O_4$. On treatment with CH_2N_2, it furnished a monoester (**2a**) which, on acetylation, gave a monoester diacetate (**2b**).

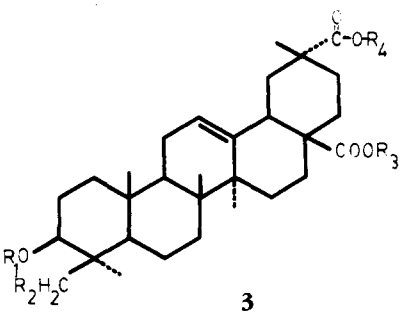


	R_1	R_2	R_3	R'_1	R'_2
1	H	OH	H	H	H
1a	H	OH	CH_3	H	CH_3
1b	Ac	OAc	CH_3	Ac	CH_3



2

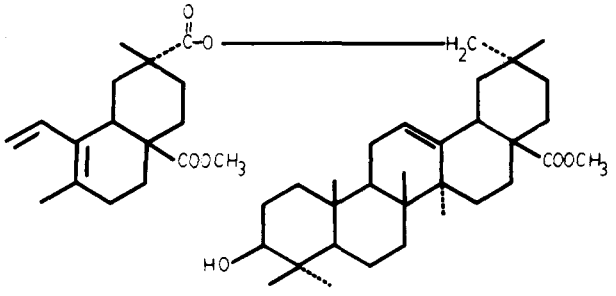
	R_1	R_2	R_3
2	H	H	OH
2a	H	CH_3	OH
2a	Ac	CH_3	OAc



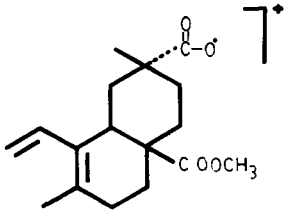
3

	R_1	R_2	R_3	R_4
3	H	OH	H	H
3a	H	OH	CH_3	CH_3
3b	Ac	OAc	CH_3	CH_3

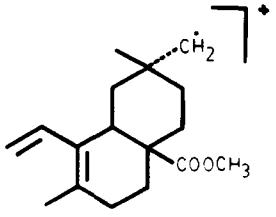
From ^{1}H-nmr and ms data the structure of **2** was deduced to be 3β,29-dihydroxyolean-12-en-28-oic acid, which has previously been isolated from *Rhipsalis mesembryanthemoides* and named mesembryanthemoidigenic acid (2). This structure was also supported from the ^{13}C-nmr spectrum of **2a** in $CDCl_3$ (Table 1). Peaks at 78.92, 122.69, and 143.44 ppm are due to C-3, C-12, and C-13, respectively. A peak at 74.33 ppm is ascribed to C-29. This peak is inverted in the gated spin echo (GASPE) spectrum, indicating that this peak is due to a methylene carbon bearing a primary OH group.



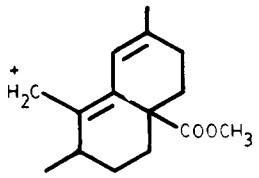
4 *m/z*=760.69 (4.34%)



5 *m/z*=291 (1.70%)



6 *m/z*=261 (39.84%)



7 *m/z*=233 (37.07%)

Compound **3** was isolated from the fractions eluted with C_6H_6-EtOAc (1:4), which crystallized from MeOH as small colorless crystals. Its mass spectrum showed a molecular ion peak at *m/z* 502, corresponding to the formula $C_{30}H_{46}O_6$. Its ir spectrum in KBr revealed the presence of hydroxyl (3425 cm^{-1}), carboxyl (1692 cm^{-1}), and trisubstitute double bond(s) (825 cm^{-1}). Its uv spectrum had a maximum at 210 nm (end absorption). Methylation with CH_2N_2 afforded a dimethyl ester (**3a**). The ^{1}H-nmr spectrum of **3a** showed five tertiary methyl signals at δ 0.66, 0.83, 1.10 (each 3H, s, 26, 25 and 27-Me groups, respectively), 1.22 (6H, s, 23 and 30-Me), a single proton double doublet centered at 2.88 (J=5, 13.75 Hz) characteristic of the H-18 proton of an olean-12-en-system (3), a multiplet centered at δ 3.35 attributed to H-3 and H-24, two carbomethoxy signals at δ 3.60 and 3.65, and a triplet at δ 5.29 (1H, J=3.75 Hz) ascribable to the proton at H-12, which is commonly encountered in the triterpenes of the oleanene and ursene series. Acetylation of **3a** with Ac_2O and pyridine furnished a dimethyl ester diacetate (**3b**). Its ir spectrum showed no OH absorption. The mass spectrum of **3** revealed the characteristic fragmentation of the ring C of Δ^{12} amyrin derivatives with a base peak at *m/z* 278 and a peak at *m/z* 223 corresponding to ions 10 and 11 due to RDA fragmentation (4). The former peak is shifted at 306 in **3a** and remains unchanged in **3b**, indicating the presence of two carboxyl groups in ring D/E and two hydroxyl groups in ring A/B.

The ^{1}H-nmr spectrum of **3b** showed two acetoxy methyl signals at δ 2.02 and 2.04; a triplet centered at δ 4.57 (1H, J=8 Hz) is due to the H-3α. The chemical shift and coupling pattern of this proton suggested that one OH group is located at C-3 and oriented in the β configuration. The ^{1}H-nmr spectrum of **3b** also showed an AB quartet centered at δ 4.25 (J=12.5 Hz) due to two methylene protons on a carbon atom bearing a primary acetoxyl group attached to an asymmetric center C-4. The chemical shift attributed to the methylene proton is within the range for an axial CH_2OAc (5). These data establish the orientation of the two hydroxyl groups in compound **3** as 3β and 24. In the ^{1}H-nmr spectrum of **3b**, the highest C-methyl signal (δ 0.71) appeared upfield from δ 0.775 and one of the two methyl ester signals is at δ 3.60, suggesting the presence of a C-28 carboxyl group in the compound (6). The possible point of attachment of the other carboxyl group is at C-20. The influence of substitution on methyl frequencies in the ^{1}H-nmr spectrum of 12-oleanene derivatives was applied in order to

TABLE 1. ^{13}C-nmr Chemical Shifts (in ppm) of Compounds **1a, 2a,** and **3a** in $CDCl_3$

Carbon number	1a[a]	2a	1a	3a	Carbon number	1a[a]	2a	1a	3a
1	38.74	38.74	38.53	38.40	17	46.76	46.96	46.44	46.41
2	27.61	27.60	27.61	27.54	18	40.31	40.42	40.13	40.09
3	78.92	78.92	80.59	80.82	19	37.04	40.05	39.80	39.64
4	38.26	38.40	42.60	42.14	20	34.62	35.74	42.60	42.71
5	55.35	55.18	55.90	55.80	21	29.66	28.22	31.22	31.15
6	18.38	18.28	18.38	18.37	22	31.37	31.48	31.22	31.15
7	32.69	32.61	32.91	32.82	23	28.13	28.05	19.37	19.33
8	39.33	39.25	39.33	39.26	24	15.60	15.52	64.40	64.44
9	47.66	47.59	47.66	47.63	25	15.28	15.25	15.84	15.82
10	37.04	37.01	36.74	36.71	26	16.82	16.79	16.71	16.69
11	23.44	23.37	23.13	23.08	27	26.04	25.95	26.04	25.83
12	122.94	122.69	123.23	123.15	28	178.02	178.03	177.65	177.68
13	143.22	143.44	142.72	142.75	29	74.18	74.33	177.91	178.77
14	41.54	41.56	41.54	41.52	30	19.52	18.97	22.53	22.38
15	27.16	27.14	28.37	28.21	OMe	51.65	51.53	51.65	51.66
16	23.13	23.04	23.66	23.64	OMe			51.65	51.85

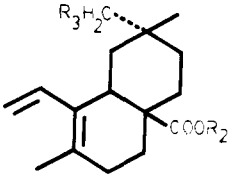
[a]These values refer to the carbon numbers 1′, 2′ etc.

determine the orientation of the carboxyl group at C-20 (7) in **3**. The observed chemical shifts are in better agreement with those calculated for α carbomethoxy and β methyl groups attached to C-20 rather than β-carbomethoxy and α-methyl groups. Consequently, the structure and stereochemistry of **3** is established as 3β,24-dihydroxyolean-12-en-28,29-dioic acid. This proposed structure was also supported by the ^{13}C-nmr spectrum of **3a** (Table 1), which showed signals for the double bond at 123.15 (C-12), 142.75 (C-13) ppm, close to the values in Δ^{12} oleanene series of triterpenoids. There were two carbon atoms bearing oxygen functions at 80.82 (C-3) and 64.44 ppm (C-24). The latter peak was inverted in the GASPE spectrum indicating that this carbon atom is a methylene, bearing a primary hydroxyl group.

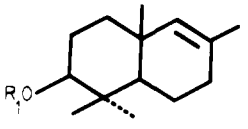
The molecular formula of compound **1**, $C_{60}H_{92}O_9$, suggested that this compound is an ester of compounds **2** and **3**. In the ^1H-nmr spectrum of **1a** the signal due to the 29-hydroxymethylene proton was shifted to relatively low field at δ 3.69 as compared to this signal in the ^1H-nmr of **2a** at δ 3.25. This low field shift suggested that the 29-hydroxymethylene proton of **2** is esterified with the carboxylic group of **3**. On alkaline hydrolysis, **1** was easily and completely converted into **2** and **3**. This ease of hydrolysis suggested that the 29-COOH (equatorial), rather than the more sterically hindered 28-COOH group, is involved in the ester formation. Thus, the ester linkage is between the 29-carboxylic group of **3** with the 29-hydroxyl group of **2**. The ease of hydrolysis also confirmed the position of the carboxylic group in compound **3** at C-29, because a C-30 carboalkoxy group would not be easily hydrolyzed.

The low resolution mass spectrum of **1a** also supported the proposed structure. It showed a molecular ion peak at *m/z* 984.8 (calcd for $C_{62}H_{96}O_9$, 984.7). A peak of *m/z* 760.6 is due to structure **4** (calcd for $C_{48}H_{72}O_7$, 760.5). There is a peak at *m/z* 261 due to structure **6**. The base peak at *m/z* 201 arose due to the removal of $HCOOCH_3$ from **6**. The other RDA fragment, **5**, easily lost CO_2, $(CO_2+COOCH_3)$, and $(CO_2+HCOOCH_3)$ giving rise to strong peaks at *m/z* 247, 188, and 187, respectively.

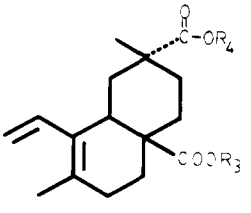
On the basis of the above spectra, structure **1** was suggested to be an ester of 3β,24-dihydroxyolean-12-en-28,29-dioic acid (**3**) and 3β,29-dihydroxyolean-12-en-28-oic



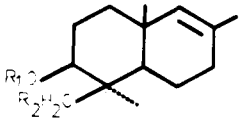
	R_2	R_3
8	H	OH
8a	CH_3	OH
8b	CH_3	OAc



9 R_1=H



	R_3	R_4
10	H	H
10a	CH_3	CH_3



	R_1	R_2
11	H	OH
11a	Ac	OAc

acid (**2**). This structure was also supported by a comparison of the ^{13}C-nmr spectral data of **1a** with those of **2a** and **3a** (Table 1).

In order to determine whether the ester of these two compounds (**2** and **3**) might have been formed during acidic hydrolysis of the saponins into sapogenins, a mixture of **2** and **3** was refluxed with methanolic HCl under the same conditions used for acidic hydrolysis of the crude saponins. It was found that mixtures of **2** and **3** do not yield the ester **1** under these conditions. This indicates that compound **1** is a genuine sapogenin and not an artifact formed during acidic hydrolysis of the saponins.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The equipment used, the plant material, the isolation of saponins, acidic hydrolysis, and chromatography of the sapogenin mixture have been described in an earlier paper (1). Tlc was carried out on silica gel plates using the following solvent system: (a) $CHCl_3$-MeOH (9:1), (b) C_6H_6-EtOAc (9:1), and (c) C_6H_6-EtOAc (7:3).

OFFICIGENIN (**1**).—Compound **1** was eluted from the silica gel column with $CHCl_3$-MeOH (9:1), crystallized from Me_2CO as colorless crystals (400 mg), mp, 264-265°, [α]D +32.30 (C=0.19, MeOH), uv λ max (MeOH) 212 nm (end absorption); ir ν max (KBr) 3440 (OH), 2940 (CH_2), 1728 (ester), 1705 (CO, acid), 1468, 1385, 1250, 1115, 1025, 995, 825 and 720 cm^{-1}. *Anal.* calcd for: $C_{60}H_{92}O_9 \cdot H_2O$; C, 73.88; H, 9.72. Found: C, 73.31; H, 9.54.

METHYLATION OF **1**.—Compound **1** (100 mg) was dissolved in MeOH and treated with an ethereal solution of CH_2N_2 at room temperature for 30 min. After evaporation of the solvent from the reaction mixture, the dimethyl ester (**1a**) was crystallized from MeOH as colorless crystals (95 mg), mp, 169°; ^{1}H-nmr (100 MHz, $CDCl_3$) δ 0.66, 0.69, 0.75, 0.83, 0.88 (each 3H, s, 5×CH_3), 0.96, 1.09, 1.22 (each 6H, s, 6×CH_3), 2.88 (2H, unresolved dd, H-18 and H-18′), 3.25 (m, H-3, H-3′, and H-24), 3.61 (6H, s, 2×$COOCH_3$), 3.69 (2H, s, H-29′), 5.28 (2H, t, J=3.13 Hz, H-12 and H-12′); ^{13}C-nmr see Table 1; ms *m/z* 984.8 (0.05, M^+, calcd for $C_{62}H_{96}O_9$, 984.7), 983.75 (0.05), 982.7 (0.1), 966.8 (1.0, M^+-H_2O), 948.8 (1.2, M^+-2H_2O), 934 (2.0), 907 (0.5), 891 (0.7), 875 (0.8), 776 (1.4), 761 (2.3), 760.69 (4.3, **4**, calcd for $C_{48}H_{72}O_7$, 760.5), 742 (2, **4**-H_2O), 728 (1.1), 716 (1), 700 (1.2), 684 (1.5), 606 (1.2), 552 (0.5), 520 (1.2), 493 (1.8), 468 (7.8), 455 (4.4), 437 (4.2), 435 (3.6), 409 (8.2), 407 (9.2), 391 (6.8), 379 (3.2), 313 (4.0), 293 (9.4), 292 (2.9), 291 (1.7, **5**), 279 (11.2), 261 (49.9, **6**), 260 (39), 255 (11.2), 247 (75.7, **5**-CO_2), 233 (37, **7**), 203 (50.60), 201 (100, **6**-$HCOOCH_3$), 189 (54), 188 (34.4, **5**-CO_2+$COOCH_3$), 187 (84.2, **5**-CO_2+$HCOOCH_3$), 173 (57.2), 161 (42.2), 145 (54.7), 135 (47.5), 133 (55.4), 131 (51.6), 119 (61.9), 105 (60.8), 95 (74.3), 81 (77.6), 54.7 (57). *Anal.* calcd for: $C_{62}H_{96}O_9 \cdot 3H_2O$; C, 71.63; H, 9.89. Found: C, 71.8; H, 9.6.

ACETYLATION OF **1a**.—Compound **1a** (30 mg) was treated with Ac_2O (15 ml) in pyridine (1 ml) at room temperature overnight. Ice was added to the reaction mixture, whereby a white precipitate was obtained. The precipitate was filtered, washed with H_2O, and dried. It was crystallized from MeOH as white crystals of dimethyl ester triacetate **1b** (25 mg), mp, 157°; ^{1}H-nmr (100 MHz, $CDCl_3$); δ 0.72 (6H, s, 2×CH_3), 0.86 (6H, s, 2×CH_3), 0.94 (3H, s, 1×CH_3), 0.98 (3H, s, 1×CH_3), 1.01 (3H, s, 1×CH_3), 1.13 (6H, s, 2×CH_3), 1.25 (3H, s, 1×CH_3), 1.27 (3H, s, 1×CH_3), 2.03, 2.04, 2.05 (each 3H, s, 3×$OCOCH_3$), 2.9 (2H, dd, J=13.75, 5 Hz, H-18 and H-18′), 3.63 (6H, s, 2×$COOCH_3$), 3.73 (2H, s, H-29′), 4.25 (2H, ABq, J=12.5 Hz, H-24), 4.5 (2H, t, J=8 Hz, H-3, H-3′), 5.3 (2H, t, J=3.25 Hz, H-12 and H-12′); no molecular ion, fragmentation at *m/z* 1050 (1.5 M^+-AcOH+H), 991 (1.5, M^+-2AcOH+H), 931 (1.2 M^+-3AcOH+2H), 866 (0.5), 800 (2), 742 (3), 683 (0.5), 603 (2), 511 (2), 495 (2), 481 (2), 451 (10), 391 (5), 375 (3), 313 (5), 292 (5), 279 (6), 273 (6), 261 (22), 247 (42), 201 (96), 187 (100), 173 (74), 159 (68), 145 (68), 133 (54), 119 (54), 104 (47), 95 (40), 81 (44), 67 (38), 57 (78).

ALKALINE HYDROLYSIS OF **1**.—Compound **1** (200 mg) was refluxed with 10% (w/v) NaOH in 70% MeOH, for 4 h; the MeOH from this reaction mixture was evaporated and acidified with HCl. The reaction mixture was precipitated by adding H_2O. The precipitate was filtered and washed with H_2O. It showed two spots on tlc (silica gel; solvent a). It was chromatographed on a silica gel column which was eluted with a solvent gradient of increasing polarity of C_6H_6 and EtOAc to afford **2** and **3**.

3β,29-DIHYDROXYOLEAN-12-EN-28-OIC ACID (**2**).—The fractions eluted with C_6H_6-EtOAc (1:1) yielded white crystals on evaporation which were then recrystallized from MeOH as colorless sharp needles (120 mg); mp, 302° [α]D +70.42 (C=0.3, MeOH) lit. mp, 305-309°, [α]D +70.7; ir max (KBr) 3420 (OH), 2930 (CH_2), 1695 (CO, acid), 1465, 1385, 1200, 1045, 1030, 825 (s) (trisubstituted double bond) cm^{-1}; ms *m/z* 472 (3, M^+), 455 (3), 441 (2), 316 (2), 264 (48, RDA, **8**) 233 (100, **8**-CH_2OH), 219

(10), 207 (23, RDA, **9**), 201 (46), 187 (11), 175 (8), 159 (10), 147 (8), 133 (8), 119 (14), 105 (13), 95 (12), 81 (14), 69 (14), 49 (16). *Anal.* calcd for: $C_{30}H_{48}O_4$: C, 76.32; H, 10.23. Found: C, 76.20; H, 10.40.

METHYLATION OF **2**.—Compound **2** (50 mg) was methylated as **1** to yield the monomethyl ester (**2a**). It was crystallized from MeOH as colorless needles (48 mg), mp, 210°, lit. mp, 213.5-214.5°; ms *m/z* 486 (3, M^+), 455 (6), 278 (16, RDA, **8a**), 247 (100, **8a**-CH_2OH), 207 (16, RDA, **9**), 201 (34). The ^{1}H-nmr data are identical as those published in literature (2).

ACETYLATION OF **2a**.—Compound **2a** (20 mg) was acetylated and worked up as described above in the case of **1** to yield monomethyl ester diacetate (**2b**). It was crystallized from MeOH as fine colorless crystals (15 mg) mp, 225°; ms *m/z* 570 (3, M^+), 510 (7), 451 (2), 320 (30, RDA, **8b**), 247 (100, **8b**-CH_2OAc), 201 (74).

3β,24-DIHYDROXYOLEAN-12-EN-28,29-DIOIC ACID (**3**).—Compound **3** was isolated from the fractions eluted with C_6H_6-EtOAc (1:4) and purified by recrystallization from MeOH as small colorless crystals (50 mg), mp, 290°, [α]D +66.66 (C=0.03, MeOH), uv λ max (MeOH) 210 nm; ir ν max (KBr) 3425 (OH), 2925 (C-H), 1692 (CO, acid), 1470, 1380, 1255, 1120, 1045, 1025, 825 (s) (trisubstituted double bond) cm^{-1}; ms *m/z* 502 (1, M^+), 466 (1, M^+-$2H_2O$), 453 (1, M^+-H_2O-CH_2OH), 425 (1), 412 (1, M^+-2COOH), 394 (1, 412-H_2O), 379 (2), 340 (1), 332 (2), 316 (1), 286 (2), 278 (100, RDA, **10**), 260 (9), 233 (35, **10**-COOH), 223 (15, RDA, **11**), 207 (16), 187 (**10** -2COOH+H), 189 (18, 233-CO_2), 175 (46, 189-CH_2), 159 (18), 144 (13), 131 (11), 119 (19), 105 (28), 92 (18), 81 (22), 69 (12), 55 (22).

METHYLATION OF (**3**).—Compound **3** (40 mg) was methylated in the usual manner as described above to yield the dimethyl ester (**3a**), which was crystallized from MeOH as colorless crystals (35 mg), mp, 200°, [α]D +30.3 (C=0.07 $CHCl_3$); ^{1}H-nmr (100 MHz, $CDCl_3$), δ 0.66 (3H, s, 26-Me), 0.83 (3H, s, 25-Me), 1.10 (3H, s, 27-Me), 1.22 (6H, s, 23 and 30 Me), 2.88 (1H, dd, *J*=5, 13.75 Hz, H-18), 3.35 (m, H-3 and H-24), 3.60, 3.65 (each 3H, s, 2×$COOCH_3$), 5.29 (1H, t, *J*=3.75 Hz, H-12); ms *m/z* 530 (5, M^+), 512 (1, M^+ -H_2O), 499 (2, M^+ -CH_2OH), 481 (2), 471 (5, M^+ -$COOCH_3$), 453 (1), 393 (1), 360 (1), 306 (50, RDA, **10a**), 301 (1), 292 (10), 274 (4), 259 (2), 247 (46, **10a**-$COOCH_3$), 233 (12, 247-CH_2), 223 (10, RDA, **11**), 215 (22), 205 (12), 188 (18, **10a**-2 $COOCH_3$), 187 (100, **10a**-2$COOCH_3$+H), 175 (28), 174 (22), 159 (12), 144 (9), 131 (10), 119 (12), 105 (16), 92 (15), 81 (18), 69 (10), 55 (17).

ACETYLATION OF **3a**.—Compound **3a** (15 mg) was acetylated as **1** to yield **3b**, which was crystallized from MeOH as white crystals of dimethyl ester diacetate (**3b**) (12 mg), mp, 141°; ^{1}H-nmr (100 MHz, $CDCl_3$), δ 0.71 (3H, s, 26-Me), 0.93 (3H, s, 23-Me), 1.00 (3H, s, 25-Me), 1.12 (3H, s, 27-Me), 1.24 (3H, s, 30-Me), 2.02, 2.04 (each 3H, s, 2×$OCOCH_3$), 2.9 (1H, dd, *J*=5, 13.75 Hz), 3.60, 3.66 (each 3H, s, 2×$COOCH_3$), 4.25 (2H, ABq, *J*=12.5 Hz, H-24), 4.57 (1H, t, *J*=8 Hz, H-3), 5.3 (1H, t, *J*=3.75 Hz, H-12); ms *m/z* 614 (1, M^+), 554 (6, M^+-AcOH+H), 495 (2, 554-$COOCH_3$), 435 (4, 495-AcOH+H), 307 (14, RDA, **11a**), 306 (36, RDA, **10a**), 292 (18, **10a**-CH_2), 247 (47, **10a**-$COOCH_3$), 232 (14, 247-CH_3), 215 (24), 187 (100, **10a**-2$COOCH_3$+H), 174 (22), 119 (12), 81 (14), 55 (10).

ATTEMPTED ESTERIFICATION OF **2** WITH **3**.—A mixture (40 mg) of compound **2** and **3** was refluxed with methanolic HCl (10 ml MeOH, 1 ml H_2O, 1.7 ml HCl) for 5 h. The MeOH from this reaction mixture was evaporated and H_2O added, which furnished a white precipitate. It was filtered and dried. It showed identical spots (silica gel, solvent a) with that of the original mixture of **2** and **3**. No spot corresponding to **1** was detected.

ACKNOWLEDGMENTS

The authors thank the University Grants Commission of Pakistan for financial support of this work.

LITERATURE CITED

1. V.U. Ahmad, N. Bano, and S. Bano, *Phytochemistry*, (in press).
2. B. Tursch, J. Leclercq, and G. Ghiurdoglu, *Tetrahedron Lett.*, 4161 (1965).
3. T. Cheung and T.C. Yan, *Aust. J. Chem.*, **25**, 2003 (1972).
4. H. Budzikiewicz, J.M. Willson, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 3688 (1963).
5. L. Canonica, B. Danieli, P. Manitto, G. Russo, E. Bombardelli, and A. Bonati, *Gazz. Chim. Ital.*, **98**, 1712 (1968).
6. M. Shamma, R.E. Glick, and R.O. Mumma, *J. Org. Chem.*, **27**, 4512 (1962).
7. B. Tursch, R. Savoir, R. Ottinger, and G. Ghiurdoglu, *Tetrahedron Lett.*, 539 (1967).

Received 25 April 1984