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Diterpenes from the marine mangrove *Bruguiera gymnorhiza*

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Abstract

Steviol and five new diterpenes have been isolated from the outer layer of the root bark of *Bruguiera gymnorhiza* Lam of the Andaman and Nicobar Islands. They are *ent*-kaur-16-en-13-hydroxy-19-al; 15(*S*)-isopimar-7-en-15,16-diol, *ent*-kaur-16-en-13,19-diol, methyl-*ent*-kaur-9(11)-en-13,17-epoxy-16-hydroxy-19-oate; 1β,15(*R*)-*ent*-pimar-8(14)-en-1,15,16-triol. Their structures were established by means of spectral studies, chemical reactions and, in case of the last compound, by X-ray analysis.

Keywords: *Bruguiera gymnorhiza*; Rhizophoraceae; Diterpenes; Steviol

1. Introduction

Bruguiera gymnorhiza is a large evergreen tree found in the mangrove forests of India (Hooker, 1982). Previous examination of *B. gymnorhiza* showed the presence of steroids, triterpenes, hydrocarbons and wax esters from its leaves (Sarkar & Ganguly, 1978; Misra, Choudhury, Dutta, & Ghosh, 1984; Ghosh, Misra, Dutta, & Choudhury, 1985; Misra, Dutta, Chattopadhyay, Choudhury, & Ghosh, 1987), and gibberellins from its fruits (Ganguly & Sircar, 1974). As part of our investigations on marine sources for bioactive compounds we have examined the outer layer of the root bark of *B. gymnorhiza* collected from the Andaman and Nicobar islands and the results are presented in this paper.

2. Results and discussion

The outer layer of the root bark was air-dried, powdered and the powdered material was extracted successively with *n*-hexane and ethanol. Column chromatography of the residues from the *n*-hexane and the tannin-freed alcohol extracts furnished six diterpenes **1**–**6** (**1**–**5** from the hexane extract and **1**–**6** from the ethanol extract) of which compound **1** was identified as steviol, the aglycone of the sweet glycosides of *Stevia rebaudiana* (Mosettig & Nes, 1955). The identification of **1** was achieved by means of its physical and spectral data, chemical reactions and comparison with literature data (Mosettig & Nes, 1955; Bearder, MacMillan, Wels, & Phinney, 1975; Bearder et al., 1976; Yamasaki, Kohda, Kobayashi, Kasai, & Tanaka, 1976; Hanson, Siverns, Piozzi, & Savone, 1976; Kohda, Kasai, Yamasaki, Murakami, & Tanaka, 1976; Orihara, Saiki, & Furuya, 1991).

NMR data of compounds **1**, **2** and **4** (Tables 1 and 2) revealed that they differed only at C-19. While compound **1** had a C-19 carboxyl group, compounds **2** and **4** had a C-19 CHO and C-19 CH_2OH group, respectively. This was confirmed by the following reactions.

Jones oxidation of **2** furnished **1** and CrO_3-pyridine oxidation of **4** gave **2**. Sodium borohydride reduction of **2** gave **4** which was also obtained by the LAH reduction of **1**. Thus **2** and **4** were *ent*-kaur-16-en-13-hydroxy-19-al and *ent*-kaur-16-en-13,19-diol, respectively. The cooccurrence of diterpenes representing the three oxidation states of the C-19 methyl group, e.g. the triad *ent*-kaur-16-en-19-oic acid, *ent*-kaur-16-en-19-al and *ent*-kaur-16-en-19-ol, is quite common in natural sources (Connolly & Hill, 1991), even though the cooccurrence of the steviol series (**1**, **2** and **4**) is encountered for the first time in the present study. The observed ^1H and ^{13}C NMR spectra of compounds **2** and **4** (Tables 1–2) were as expected for the CHO and CH_2OH variants of the C-19 COOH group of

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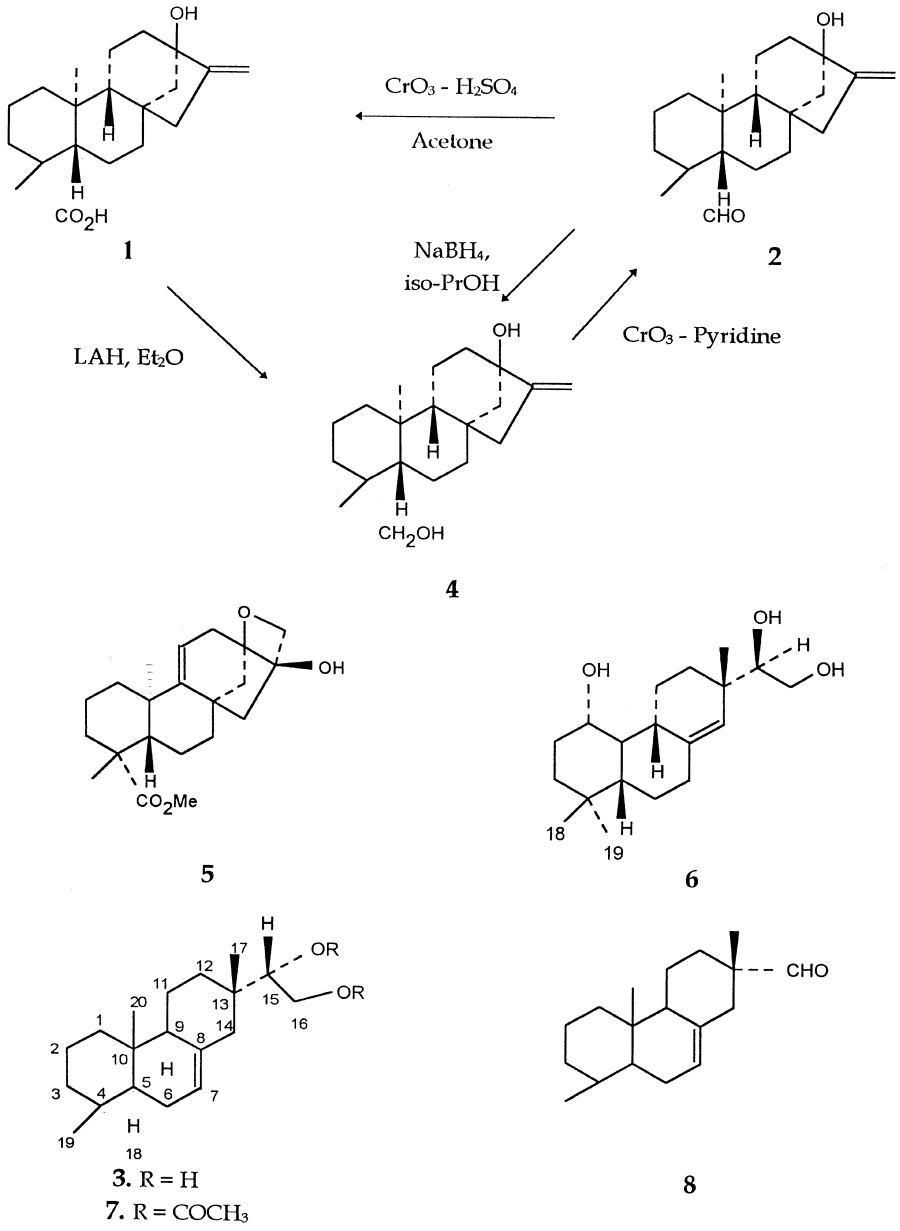


1. The ^{13}C NMR spectra of compounds **2** and **4** showed similar changes from the spectrum of steviol **1** as found in the naturally occurring C-19 oxidation series (Wu et al., 1996).

Compound **3** analyzed for $C_{20}H_{34}O_2$ and gave a diacetate **7**. It underwent ready oxidation with $NaIO_4$ to give an aldehyde (**8**) with one carbon less than the parent compound, indicating that the two hydroxyl groups were present as a 1,2-dihydroxyethyl group in the molecule.

The ^1H NMR spectrum of compound **3** Table 1 showed the presence of a trisubstituted double bond, the AB_2 system of the 1,2-dihydroxyethyl group and four tertiary methyl groups. The ^{13}C NMR spectrum of compound **3** Table 2 showed signals characteristic of a Δ^7-isopimarane diterpene (Wenkert & Buckwalter, 1972) having a 1,2-dihydroxyethyl group at C-13. With the exception of the signals due to the 1,2-dihydroxyethyl group which were observed at δ 73.0 d, and 62.3 t, other signals in compound **3** were very close to the values observed for 7,15-isopimaradiene (Wenkert & Buckwalter, 1972). Thus compound **3** was isopimar-7-en-15,16-diol. The configuration at C-15 may be assigned on the basis of its carbon chemical shift.

The C-15 epimers of Δ^{14}-isopimaren-15,16-diols, were found to show different chemical shifts; the 15(*R*)-isomer at δ 78.2 and the 15(*S*)-isomer at δ 75.5 (Wenkert, Ceccherelli, Raju, Polonsky, & Tingoli, 1979). In case of compound **3**, the C-15 had chemical shift of δ 73.0. On

Table 1
^{1}H NMR data of compounds **1**–**6**

				4				**6**	
H	**1** 90 MHz	**2** 90 MHz	**3** 400 MHz	400 MHz (CD_3OD)	90 MHz	90 MHz (pyridine-d_5)	**5** 90 MHz	400 MHz	90 MHz (pyridine-d_5)
1								δ 3.46 t, J=10.0 Hz	3.55 t, J=10.0 Hz
7			5.47 dd, J=5.5 and 2.2 Hz						
11							5.34 t		
14								5.32 s	5.75 s
15			3.66 dd, J=10.9 and 2.9 Hz					3.71 d, J=10.3 Hz	3.95 m (3H)
16			3.70 dd, J=9.8 and 2.9 Hz 3.49 dd, J=10.9 and 9.8 Hz					3.67 d, J=9.7 Hz 3.51 dd, J=10.3 and 9.5 Hz	
17	5.03 br (1H)	5.05 br (1H)	0.88 s	4.92 s (1H)	5.05 br	5.40 br	3.55 d, J=12.0 Hz	0.89 s	1.15 s
	4.80 br (1H)	4.85 br (1H)		4.75 s (1H)	4.80 (1H)	4.95 br	3.62 d, J=12.0 Hz		
18	1.25 s	1.02 s	0.96 s	1.00 s	1.05 s	1.15 s	1.20 s	0.83 s	0.72 s
19		9.75 s	0.93 s	3.63 d, J=11 Hz (1H) 3.25 d, J=11 Hz (1H)	3.70 d, J=11 Hz (1H) 3.45 d, J=11 Hz (1H)	3.90 d, J=11 Hz (1H) 3.55 d, J=11 Hz (1H)	3.70 s ($COOCH_3$)	0.84 s	0.74 s
20	0.95 s	0.90 s	0.81 s	0.91 s	1.0 s	0.95 s	0.90 s	0.87 s	0.92 s

Table 2
^{13}C NMR data of compounds **1**–**6**

				4			**6**	
C	**1** 22.40 MHz	**2** 22.40 MHz	**3** 100 MHz	22.40 MHz (pyridine-d_5)	100 MHz (CD_3OD)	**5** 22.40 MHz	100 MHz	22.40 MHz (pyridine-d_5)
1	δ 40.0	39.5	39.8	40.6	41.2	41.0	79.1	79.0
2	19.0	19.6	18.8	20.5	21.4	20.1	30.0	32.4
3	37.8	39.0	42.2	36.1	36.9	37.6	39.8	40.3
4	43.6	48.2	32.8	39.2	40.8	44.8	29.7	30.6
5	53.9	56.4	50.5	57.0	58.2	46.5	54.2	54.7
6	21.8	20.1	23.4	20.7	21.6	18.0	22.5	23.1
7	39.4	34.0	122.4	42.2	43.2	30.0	36.5	37.2
8	41.7	41.3	135.3	41.7	42.0	40.3	139.7	137.0
9	56.9	53.1	51.7	55.2	56.4	157.4	51.2	51.8
10	39.5	39.2	35.4	39.3	39.6	38.7	44.2	44.9
11	19.0	18.1	20.0	18.7	19.5	114.7	22.4	22.8
12	41.3	41.0	35.2	40.6	40.6	38.3	32.9	33.4
13	80.4	79.9	36.5	79.8	80.7	80.0	36.8	37.7
14	46.9	46.8	45.4	47.3	48.9	49.2	128.3	131.5
15	47.4	47.3	72.9	48.2	49.0	52.9	78.5	78.6
16	155.7	155.4	62.3	157.4	157.4	78.7	63.3	63.9
17	103.0	103.0	22.7	102.9	103.8	67.9	23.5	24.0
18	28.8	24.1	33.6	28.0	28.5	28.0	33.2	33.4
19	183.3	205.6	22.3	64.0	64.9	177.8	21.7	21.9
20	15.5	16.1	15.0	18.2	19.1	23.4	9.0	10.0
OCH_3						51.4		

this basis an (*S*)-configuration may be assigned for this centre; thereby making compound **3** 15(*S*)-isopimar-7-en-15,16-diol, a new diterpene.

Compound **6** analyzed for $C_{20}H_{34}O_3$ and its NMR spectra (Tables 1–2) showed that it was also a tricyclic diterpene possessing a trisubstituted double bond, a 1,2-dihydroxyethyl group and a secondary hydroxyl group. The ^{1}H NMR spetra showed an olefinic proton as a singlet, the AB_2 system of the 1,2-dihydroxyethyl group and four tertiary methyl groups Table 1. Its ^{13}C NMR spectra Table 2 showed twenty signals including the trisubstituted double bond, the 1,2-dihydroxyethyl group, a secondary hydroxyl group and four tertiary methyls. The chemical shifts of all these groups indicated that compound **6** was a 8(14)-isopimarene (Wenkert & Buckwalter, 1972) having a 1,2-dihydroxyethyl group at C-13 and another secondary hydroxyl group.

A comparison of the ^{13}C NMR spectrum of compound **6** with that of 8(14),15-pimaradiene (Wenkert & Buckwalter, 1972) showed differences expected for the presence of a hydroxyl group at C-1 and a 1,2-dihydroxyethyl group at C-13 in the former, i.e. it showed differences at C-1, C-2, C-3, C-9, C-10 and C-20 (due to the OH group at C-1) and at C-12 and C-13 (due to the 1,2-dihydroxyethyl group at C-13). Thus compound **6** was 1-hydroxy-8(14)-isopimaren-1,15,16-triol.

An X-ray analysis of compound **6** showed that it had the structure shown in the Fig. 1 and is (1β), 15(*R*)-*ent*-pimar-8(14)-en-1,15,16-triol, a new diterpene. It may be mentioned that 8(14)-pimaren-1,15,16-triol (leucophleol) (**9**) (Bansal, Garcia-Alvarez, Joshi, Rodriguez, & Patni, 1980) isolated from *Acacia leucophloea* differs from **6** in its physical and spectral data.

Compound **5** analyzed for $C_{21}H_{30}O_4$, indicating seven double bond equivalents in the molecule. Its IR spectrum showed bands at 3430 (hydroxyl) and 1702 cm^{-1} (carbonyl group). Its ^{1}H NMR spectrum Table 1 showed a trisubstituted olefinic proton, a carbomethoxy group, an oxymethylene group and two tertiary methyl groups.

The ^{13}C NMR spectrum of compound **5** Table 2 showed 21 signals as an ester carbonyl, a trisubstituted double bond, two quaternary oxygen bearing carbons, an oxymethylene carbon, a methoxy carbon, three quaternary carbons, eight methylene groups, one methine and two methyl groups. The spectrum was strikingly similar to the spectrum reported (Reynolds, Eursquez, Escobar, & Lozoya, 1984) for grandiflorenic acid (**10**) (*ent*-kaur-9(11),16-dien-19-oic acid) except at C-12, C-13, C-14, C-15, C-16, C-17. This close similarity suggested that compound **5** was a modified grandiflorenic acid.

The grandiflorenic acid skeleton accounts for six degrees of unsaturation and two of the four oxygens present in **5** and it could be modified to give **5** by the addition of two more oxygens in a suitable way. The presence of two quaternary oxygen bearing carbon atoms in **5** and its cooccurrence with compounds **1**, **2** and **4**, all of them 13-hydroxy-kaur-16-ene diterpenes, suggested that **5** too had an oxygen function at C-13. Further, the absence of the Δ^{16}-double bond and the presence of another quaternary oxygen bearing carbon suggested the presence of an oxygen at C-16 also. The chemical shift of C-17 and the remaining double bond equivalent further indicated that C-17 in compound **5** was linked to the oxygen of C-13 by an ether linkage. Thus compound **5** may be formulated as methyl-*ent*-kaur-9(11)-en-13,17-epoxy-16-hydroxy-19-oate.

The proposed structure for **5** was in complete agreement with the observed spectral data and was supported by the fact that 13-hydroxy-grandiflorenic acid (**11**) occurs naturally (Bohlmann, Jakupovic, Schuster, King,

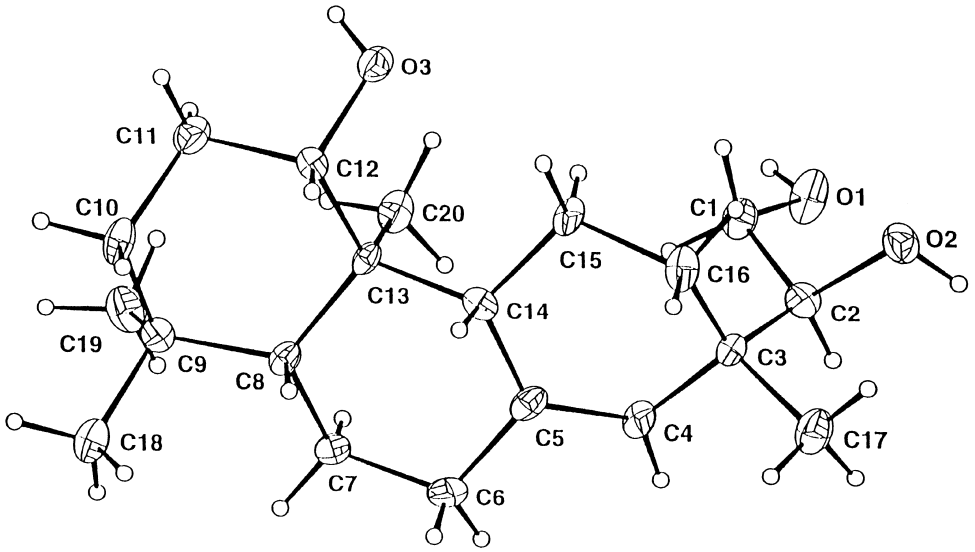
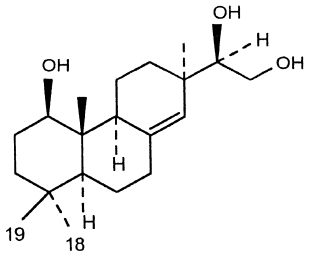


Fig. 1. X-ray structure of compound 6.



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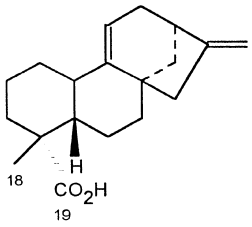

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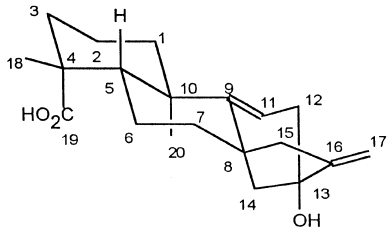

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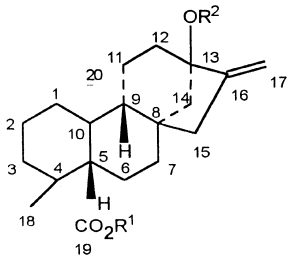

12. $R^1 = CH_3$, $R^2 = H$
13. $R^1 = H$, $R^2 = COCH_3$

& Robinson, 1982). The ^{13}C NMR spectrum of 13-hydroxy grandiflorenic acid (**11**) is not available but the observed C-12, C-13 and C-14 signals in **5** are as expected for a 13-hydroxy bearing compound. The observed C-15, C-16 and C-17 signals are also as expected for a kaureniod diterpene having a hydroxyl at C-16 and a hydroxymethyl group C-17 (Wu et al., 1996). Compound **5**, a new diterpene may arise from 13-hydroxy grandiflorenic acid (**11**) via the 16–17 epoxide which on attack by the C-13 hydroxyl could give the 13–17 cyclic ether and the C-16 hydroxyl group with the stereochemistry as depicted in structure **5**.

The cooccurrence of compounds **1**, **2** and **4** in the present mangrove has biogenetic significance. Compounds **1**, **2** and **4** represent the early stages of the 13-hydroxy gibberellin biosynthesis in higher plants (Bearder et al., 1975). Compounds **2** and **4** are new compounds and, even though **1** is a known compound, its isolation in the present study is the first report of the isolation of steviol in its free state from a natural source. So far it has been reported only as its glycosides (Crammer & Ikan, 1986).

3. Experimental

IR spectra were recorded as nujol mulls on a Perkin-Elmer 841 IR spectrometer. NMR spectra were recorded on Bruker 400 WM instrument (400 MHz for ^1H and 100 MHz for ^{13}C) or JEOL-JNM Ex 90 FT NMR spectrometer (90 MHz for ^1H and 22.40 MHz for ^{13}C) with TMS as internal standard, and in $CDCl_3$ unless otherwise stated. Carbon assignments are supported by DEPT experiments. The CI mass spectra were obtained using NH_3.

3.1. Extraction and isolation of compounds

The outer layer of the root bark of *B. gymnorhiza* was collected from Chiriatapu of the South Andaman group of islands in April 1991. The material was air-dried, powdered and the powder (4.2 kg) was exhaustively extracted with *n*-hexane and EtOH. The brown colored hexane extract (5 l) was conc. to 200 ml when a light grey colored solid (0.5 g) was precipitated. This solid was filtered and solvent was removed from the filtrate. The resulting dark red viscous residue (43 g) was subjected to CC over a column of silica gel (Acme brand, 100–200 mesh, 400 g, 3.5 cm × 75 cm) using solvents of increasing polarity from *n*-hexane through EtOAc. In all 250 frs. (300 ml fr.) were collected. Closely related frs. were combined and some of the resulting combined frs. were purified by rechromatography and fr.al crystallization. Compounds **1**–**5** were obtained from the following frs.: mixture of **1** and **2** (1.0 g) from frs. 68–85 (hexane–EtOAc, 9.5:0.5), **3** (0.5 g), from frs. 104–142 (hexane–EtOAc, 9.5:0.5); **4** (0.15 g), from frs. 188–199 (hexane–EtOAc, 6:4), and **5** (0.40 g), from frs. 200–212 (hexane–EtOAc, 4:6). Compounds **1** and **2** were sepd from the mixture by washing an ethereal soln of the mixture with aq. sodium bicarbonate soln in which compound **1** dissolved. Neutralization of the bicarbonate extract and extraction with Et_2O furnished compound **1** (0.2 g) and

removal of the solvent from the neutral Et_2O layer gave compound **2** (0.4 g).

The EtOH extract (5 l) which was dark colored, was conc. to about 750 ml and a 10% soln of neutral lead acetate in EtOH (500 ml) was added. The precipitated solid was filtered and the filtrate was saturated with hydrogen sulphide gas. The precipitated lead sulphide was filtered and solvent was removed from the filtrate. A light brown colored gum (11 g) was obtained. This was subjected to CC over a column of silica gel (200 g, 100–200 mesh, 3.5×75 cm column) with solvents from *n*-hexane through EtOAc. A total of 126 frs. (300 ml fr.) were collected from which compounds **1**–**6** were isolated after further purification from the following combined frs., **3** (0.07 g), from frs. 21–26 (hexane–EtOAc, 9:1); a mixt. of **1**, **2** and **4** (0.1 g), from frs. 34–50 (hexane–EtOAc, 8:2); **5** (0.05 g), and **6** (0.1 g) from frs. 51–62 (hexane–EtOAc, 7:3). Rechromatography of the mixture of **1**, **2** and **4** furnished a mixture of **1** and **2** from which **1** (0.03 g) and **2** (0.03 g) were separated by extraction with aq. sodium bicarbonate as in the case of the *n*-hexane extract and compound **4** (0.03 g).

3.2. Steviol (*1*)

Colorless prisms from aq. Me_2CO, mp 198° $[\alpha]^{30}_D$ −69°C (*c*, 0.06, $CHCl_3$) (lit. mp 215° $[\alpha]_D$ −94.7° (EtOH) (Mosettig & Nes, 1955); mp 199–200.5° (Bearder et al., 1975); mp 204–205° $[\alpha]_D$ −65° ($CHCl_3$) (Orihara et al., 1991)). IR ν_{max} cm^{-1}: 3600 br, 1673, 1650, 1600 and 878. R_f 0.16 (hexane–EtOAc, 8:2). ^1H and ^{13}C NMR: Tabs. 1–2. EIMS (70 eV) *m/z* (rel. int.): 318 $[M]^+$ ($C_{20}H_{30}O_3$, 42), 300 (20), 272 (6), 260 (4), 254 (7) and 121 (100).

3.3. Methyl ester (*12*)

(CH_2N_2–Et_2O), colorless prisms (from aq. MeOH), mp 110°, (Lit. mp 112–114°) (Mosettig & Nes, 1955); IR ν_{max} cm^{-1}: 3600, 1745 and 900. R_f 0.42 (hexane–EtOAc, 8:2). ^1H NMR (90 MHz): δ 0.85 s (3H, H-20), 1.20 s (3H, H-18), 3.70 s (3H, $COOCH_3$), 4.85 br (1H, H-17), 5.00 br (1H, H-17). ^{13}C NMR (22.40 MHz) (C-1 to C-20): δ 40.9, 19.4, 38.3, 44.0, 54.1, 22.1, 39.5, 41,6, 57.2, 39.5, 20.7, 41.6, 80.5, 47.3, 47.7, 156.2, 103.1, 28.9, 177.9, 15.5 and 51.3 (OCH_3).

3.4. Acetate (*13*)

(Ac_2O-pyridine, 90°, 25 h) colorless prisms (from aq. MeOH), mp 198° (lit. mp 199–201) (Bearder et al., 1976). IR ν_{max} cm^{-1}: 1730, 1690 and 900. ^1H NMR (90 MHz): δ 0.95 s (3H, H-20), 1.20 s (3H, H-18), 2,05 s (3H, $OCOCH_3$), 4.90 br (2H, H-17).

3.5. Compound *2*

Colorless needles from hexane, mp 120° $[\alpha]^{30}_D$ −59° (*c*, 0.1, $CHCl_3$). IR ν_{max} cm^{-1}: 3421 br, 1691 and 964. R_f 0.41 (hexane–EtOAc 8:2). ^1H NMR and ^{13}C NMR. Tables 1–2. EIMS (70 eV) *m/z* (rel. int.): 302 $[M]^+$ (54), 284 (17), 274 (28) and 121 (100). Found: C, 79.4; H, 9.9. $C_{20}H_{30}O_2$ requires: C, 79.5; H, 9.9%.

3.6. Jones oxidation of compound *2*

To a soln of **2** (20 mg) in Me_2CO (5 ml), CrO_3 (50 mg) and four drops of conc. H_2SO_4 were added at 0°C. The resulting mixture was stirred at room temp. for 4 h, diluted with H_2O (10 ml) and filtered. The solid on recrystallization from Me_2CO–H_2O gave a colorless solid, mp 198°C. R_f 0.16 (hexane–EtOAc, 8:2), identical with compound **1** (mmp and co-TLC).

3.7. Sodium borohydride reduction of compound *2*

Compound **2** (5 mg) was dissolved in *iso*-PrOH (5 ml) and to this soln, $NaBH_4$ (20 mg) was added. The soln was stirred at room temp. for 3 h and the reaction mixture was diluted with H_2O. The solid was filtered and recrystallization from C_6H_6–$CHCl_3$ gave a colorless solid mp 255°C, R_f 0.49 (hexane–EtOAc, 1:1) which was found to be identical with compound **4** (mmp and co-TLC).

3.8. LAH reduction of compound *1*

Compound **1** (10 mg) in dry Et_2O (5 ml) was treated with $LiAlH_4$ (50 mg). The mixture was stirred at room temp. for 3 h, diluted carefully with H_2O (10 ml) and extracted with $CHCl_3$ (2×10 ml). The $CHCl_3$ extract on evaporation of the solvent gave the product as colorless solid, mp 252–254°, R_f 0.49 (hexane–EtOAc 1:1), identical with compound **4** (mmp and co-TLC).

3.9. Compound *3*

Colorless needles from hexane, mp 150°C $[\alpha]^{30}_D$ +21° (*c*, 0.1, $CHCl_3$). IR ν_{max} cm^{-1}: 3400 br, 1620 and 905. R_f 0.35 (hexane–EtOAc 8:2). ^1H and ^{13}C NMR: Tabs. 1–2. EIMS (70 eV) *m/z* (rel. int.): 306 $[M]^+$ (52), 288 (52), 273 (36), 257 (88), 245 (65), 231 (15), 151 (27), 133 (40) and 109 (100). EIMS (4.09 eV) *m/z* (rel. int.): 307 $[M+1]^+$ (5), 306 $[M]^+$ (18), 291 (30), 288 (52), 273 (35), 257 (85), 245 (70), 164 (46), 151 (95), 149 (55), 137 (75), 133 (85), 131 (92), 121 (94), 119 (85) and 109 (100). CIMS *m/z* 324: ($C_{20}H_{34}O_2 + NH_4$, 100%). Found: C, 78.4; H, 11.1 $C_{20}H_{34}O_2$ requires: C, 78.4; H, 11.1%.

3.10. Acetylation of compound **3**

Compound **3** (100 mg) was dissolved in pyridine (2 ml) and Ac_2O (2 ml) and the reaction mixture was kept on a water bath at 80° for 6 h. Usual workup gave the acetate (**7**) as a gum. IR ν_{max} cm^{-1}: 1735. ^1H NMR (90 MHz): δ 5.35 br (1H, H-7), 5.15 m (1H), 3.70 to 4.50 m (2H), 1.95 s (3H, OAc), 2.02 s (3H, OAc), 0.92 s ($2\times CH_3$), 0.90 s ($2\times CH_3$). EIMS (70 eV) *m/z* (rel. int.): 390 $[M]^+$ ($C_{24}H_{38}O_4$, 3), 328 (22), 283 (18), 269 (54), 244 (33), 109 (90), 55 (60) and 44 (100).

3.11. Sodium periodate oxidation of compound **3**

Compound **3** (20 mg) was dissolved in MeOH (2 ml) and to this soln solid $NaIO_4$ (20 mg) was added. The soln was stirred at room temp. until the reaction was complete (3 h), diluted with H_2O (5 ml) and extracted with $CHCl_3$ (10 ml). Removal of the solvent from the dried $CHCl_3$ extract gave the product (**8**) (10 mg) as a gum. ^1H NMR (90 MHz): δ 9.55 s (1H, CHO), 5.5 br (1H, H-7), 0.85 s ($4\times CH_3$) EIMS (70 eV) *m/z* (rel. int.): 274 $[M]^+$ ($C_{19}H_{30}O$, 3), 123 (70), 109 (50), 83 (100).

3.12. Compound **4**

Colorless prisms from C_6H_6–$CHCl_3$, mp 255–257° $[\alpha]_D^{30}$ −47° (*c*, 0.1, CH_3OH). IR ν_{max} cm^{-1}: 3380 br, 1620 and 880. R_f 0.49 (hexane–EtOAc, 1:1) ^1H NMR and ^{13}C NMR: Tables 1–2. EIMS *m/z* (rel. int.): 304 $[M]^+$ (45), 273 (75), 121 (100). CIMS *m/z* (rel. int.): 322 $[M+NH_4]$ (100%). Found: C, 78.9; 11, 10.5. $C_{20}H_{32}O_2$ requires: C, 78.9; H, 10.5%.

3.13. CrO_3-pyridine oxidation of compound **4**

Compound **4** (20 mg) in pyridine (2 ml) was treated with dry CrO_3 (50 mg). The mixture was kept at room temp. overnight and the reaction mixture was diluted with H_2O (10 ml). The resulting solid was filtered and dried. The solid on recrystallization from hexane gave colorless crystals, mp 120°, identical with compound **2** (mmp Co-TLC and ^1H NMR).

3.14. Compound **5**

Colorless needles from C_6H_6–$CHCl_3$ mp 173° $[\alpha]_D^{30}$ +22 (*c*, 0.1, CH_3OH). IR ν_{max} cm^{-1}, 3430, 1702, 1620, 949 and 859. R_f 0.44 (hexane–EtOAc 1:1). ^1H and ^{13}C NMR: Tables 1–2. HR-MS: Found: *m/z* 346.2144; Calc. for $C_{21}H_{30}O_4$: *m/z*346.2142. EIMS *m/z* (rel. int.): 346 $[M]^+$ (43), 314 (65), 288 (18), 272 (39), 254 (30), 228 (44), 213 (82), 201 (33), 173 (46), 157 (39), 147 (100), 133 (42), 121 (40) and 105 (65). Found: C, 72.8; H, 8.7. $C_{21}H_{30}O_4$ requires: C, 72.8; H, 8.7%.

3.15. Compound **6**

Colorless plates from C_6H_6–$CHCl_3$ mp 220 $[\alpha]_{DD}^{30}$ −35° (*c*, 0.2, CH_3OH), IR ν_{max} cm^{-1}: 3436 br, 1631 and 973. R_f 0.37 (hexane–EtOAc 1:1) ^1H and ^{13}C NMR: Tabs. 1–2. No molecular ion in Cl and FAB spectra. EIMS *m/z* (rel. int.): 262 $[M\text{-}60]^+$ (100), 244 (29), 121 (45). Found: C, 74.5; H, 10.5. $C_{20}H_{34}O_3$ requires: C, 74.5; H, 10.6%.

3.16. X-ray structure determination of compound **6**

Crystal data; $C_{20}H_{34}O_3$, $M_r=322.47$, orthorhombic, space group $P2_12_12_1$, $a=6.944(2)$, $b=9.871(6)$, $c=26.579(9)$A (by least squares refinement of the setting angles for 250 reflections within $\theta=2.20$–$25.10°$). $V=1821.8(8)$ $Å^{-3}$, Z=4, Dc=1.176 g cm^{-3}, $T=150$ K, $\mu(MoK\alpha)=0.77$ cm^{-1}, $F(000)=712$, crystal size= $0.14\times0.12\times0.12$ mm.

Data were collected on a FAST TV Area detector diffractometer following previously described methods (Darr, Drake, Hursthouse, & Malik, 1993). From the ranges scanned, 5582 data were recorded ($2.20<\theta<25.10°$; index ranges $-7<h<7$, $-9<k<11$, $-30l<22$) and merged to give 2682 unique ($R(int)=0.0932$).

The structure was solved via direct method (Sheldrick, 1990) and refined on Fo^2 by full matrix least squares (Sheldrick, 1993) using all unique data corrected for Lorentz and polarisation factors. All nonhydrogen atoms were anisotropic. The hydrogen atoms were inserted in idealized positions with Uiso set at 1.5 times tile Ueq of the parent. The weighting scheme used was $w=1/(\sigma^2+(Fo)^2+(0.0127P)^2)$, where $P=(\max(Fo)^2+2(Fc)^2)/3$; this gave satisfactory agreement analyses. Final R_1 (on *F*) and wR_2 (on Fo^2) values were 0.1051 and 0.1085 for all 2682 data and 215 parameters. The corresponding *R*-values were 0.0501 and 0.0972 for 1167 data with $1>2\sigma(1)$. Sources of scattering factors as in Sheldrick (1993). Atomic coordinates, bond lengths and angles and thermal parameters have been deposited at the Cambridge Crystal lographic Data Centre.

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