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TRITERPENOIDS FROM *SHOREA ROBUSTA* RESIN*

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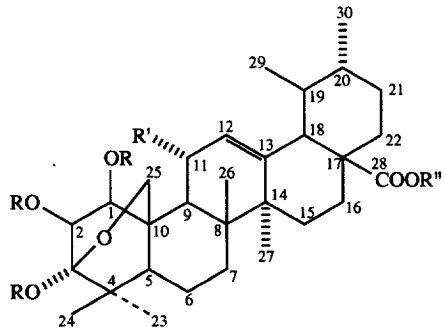
Key Word Index—*Shorea robusta*; Dipterocarpaceae; resin; triterpenoids; ursane derivatives.

Abstract—The resin of *Shorea robusta* has yielded eight known triterpenoids of which five have been isolated for the first time. It has also afforded two new triterpenoids, namely 3,25-epoxy-1,2,3,11-tetrahydroxyurs-12-en-28-oic acid and 3,25-epoxy-1,2,3-trihydroxyurs-12-en-28-oic acid whose structures have been elucidated by spectroscopic methods and chemical transformations. ©1997 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

Shorea robusta Gaertn. is a tree commonly known as sal and is famous for its strong timber leading to its maintenance under the cultivated forest throughout India. The man-made or natural cuts in the bark of stem or branches lead to the excudation of a resin which is trivially called '*Saal ki raal*'. It is produced in large quantities in India and constitutes one of the resins of commerce. It occurs in rough, brittle pieces having a faint resinous, balsamic odour and is widely used as an incense in Indian religious ceremonies as it emits copious white fumes when burnt. The resin is also reported to be used in the indigenous system of medicine as an astringent and is given for diarrhoea, dysentery, skin and ear troubles, etc. [1]. The chemical examination of its resin, tapped from the north Indian forests, has been carried out by us, the results are discussed in this paper.



1, R = R" = H, R' = OH

1a, R = Ac , R' = OAc, R" = H

1b, R = H, R' = OH, R" = Me

2, R = R' = R" = H

2a, R = Ac, R' = R" = H

2b, R = Ac, R' = H, R" = Me

RESULTS AND DISCUSSION

Shorea robusta has been the subject of several investigations, especially its seed oil [2–6]. While its wood [7] and bark [8, 9] have been examined, more emphasis is now being given to the chemistry of its resin. The oil obtained from the dry distillation of its resin has been reported to contain several mono- and sesquiterpenoids [10]. The presence of some triterpenoids have also been reported from the resin of the south Indian sal tree [11]. Recently several ursane derivatives have been isolated from the resin of east Indian sal [12, 13]. In continuation of our interest in the chemistry of Indian medicinal and aromatic plants [14–17], we now report the results of our investigations on the resin collected from north Indian sal forest.

The resin, collected by tapping from the sal forest of the Himalayan foot hills has yielded ursolic acid, α- and β-amyrin, mangiferonic acid, benthamic acid, asiatic acid, α-amyrenone and uvaol as known compounds, of which only ursolic acid, asiatic acid and α-amyrin [11, 12] have previously been reported from the resin of *S. robusta*. Mangiferonic acid and β-amyrin have been previously isolated from *S. accuminata* and *S. resina-nigra*, respectively [18]. The authenticity of the known compounds was established by the spectral data. Two new compounds, 3,25-epoxy-1,2,3,11-tetrahydroxyurs-12-en-28-oic acid (**1**) and 3,25-epoxy-1,2,3-trihydroxyurs-12-en-28-oic acid (**2**) which was

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isolated in the form of its triacetate (**2a**) were obtained from the resin of *S. robusta*.

Compound **1** showed IR bands at 3600–3200 cm^{-1} for COOH and OH along with 1693 cm^{-1} for CO, 1645 cm^{-1} for C=C, 1120, 1050 cm^{-1} for oxide. The EI-mass spectrum showed an ion at *m/z* 446 for $[M-4H_2O]^+$ and the molecular formula of **1** as $C_{30}H_{46}O_7$ was supported by the elemental analysis. The ion at *m/z* 248 as the base peak supported the C-ring cleavage through Retro–Diels–Alder fragmentation and clearly indicated that only a COOH group is present on either the D- or E-ring of a pentacyclic triterpenoid. The presence of the signals at δ 127.0 and 138.4 for C-12 and C-13, respectively, and the absence of a quartet near δ 33.0 in the ^{13}C NMR spectrum of **1** further suggested that it is an ursane derivative and not oleanane [19, 20]. Acetylation of **1** yielded **1a** having four singlets at δ 1.95, 1.99, 2.06 and 2.18 in its ^1H NMR spectrum and also four quartets at δ 21.1, 21.0, 20.8 and 20.2 in its ^{13}C NMR spectrum, clearly suggesting that **1** is a tetra-hydroxyursane. Two AB doublets appearing at δ 3.40 and 4.06 (J = 11 Hz) in $(CD_3)_2SO$ and δ 3.58 and 4.06 (J = 11 Hz) in pyridine-d_5 in the ^1H NMR of **1** remained almost unshifted in **1a** (at δ 3.52 and 3.82) further indicated the possible presence of C-O-CH_2-C- in the molecule. The ^1H NMR spectrum of **1** showed three doublets δ 4.01, 4.12 and 4.30 when recorded in $(CD_3)_2SO$. These relatively down field values of CHOH suggested that hydroxyls are attached in a single ring possibly at C-1, C-2 and C-3 positions. The ^{13}C NMR of **1a** showed a singlet at δ 101.2 suggesting that a hemiketal system existed as 3-hydroxy-3,25-epoxy in **1** which is comparable with the similar compounds isolated from *L. camara* [21]. Since C-25, forming the epoxide linkage with C-3, is α-oriented in the ursane skeleton, the hydroxyl group at C-3 was obviously given in β-disposition. The low J value (4.0 Hz) of H-1 and H-2 at δ 5.06 and 4.96 in **1a** clearly suggested that both of the hydroxyls are β-oriented, which is comparable with the similar compounds available in the literature [22]. The presence of a doublet at δ 5.10 (J = 3.5 Hz) suggested that one of the hydroxyls is attached at C-11. With the J = 3.5 Hz for H-11 and H-12 it was inferred that OH at C-11 is of α-orientation [23]. On treatment with CH_2N_2 **1** gave **1b**, which showed an additional singlet at δ 3.45 for COOMe confirming the presence of a COOH group in **1**. In the ursane skeleton normally the C-28 is oxygenated to COOH, which is supported by the presence of the typical signals at δ 52.7 for C-18 in the ^{13}C NMR and a doublet (J = 10.0 Hz) at δ 2.45 in ^1H NMR of **1** [20]. The data given above support the identification of compound **1** as 3,25-epoxy-1,2,3,11-tetrahydroxyurs-12-en-28-oic acid.

Similarly, compound **2** when acetylated gave **2a** showing a similar ^1H NMR spectrum to that of **1a** except that the doublet of **1a** at δ 5.27 appeared as a triplet (J = 7.0 Hz) at δ 5.20 in **2a** and it showed three singlets at δ 1.95, 2.00 and 2.05, which clearly suggested that **2** was a trihydroxy compound with no hydroxyl at C-11. The presence of two downfield doublets (J = 4.0 Hz) at δ 5.06 and 5.10 and the AB doublets (J = 11.0 Hz) at δ 3.55 and 3.85 were in agreement with the structure of **2a** as 3,25-epoxy-1,2,3-triacetoxyurs-12-en-28-oic acid. The conversion of **2a** to **2b** and its IR, MS, and ^{13}C NMR data (Experimental) also supported the above structure for **2a**.

EXPERIMENTAL

Strips were cut in the bark of sal trees in the Chorgalya forest of Haldwani district of Uttar Pradesh in May 1991 and resin was collected in October 1991. The forest is maintained by the U.P. Government under the Department of Forests. The dry resin (120 g) after extraction with hexane–EtOAc–MeOH (1:1:1) afforded 55 g extract. CC of the extract over silica gel yielded 26 frs with the following eluants: Frs 1 and 2 in hexane, frs 3–9 in hexane–EtOAc (19:1), frs 10,11 in hexane–EtOAc (9:1), frs 12–14 in hexane–EtOAc (3:1), frs 15 and 16 in hexane–EtOAc (1:1), frs 17 and 18 in hexane–EtOAc (1:3), frs 19 and 20 in EtOAc, frs 21 and 22 in EtOAc–MeOH (19:1), frs 23 and 24 in EtOAc–MeOH (9:1), fr. 25 in EtOAc–MeOH (4:1) and fr. 26 in EtOAc–MeOH (7:3).

Frs 1–3 were not found interesting, while fr. 4 on further CC and TLC over silica gel yielded α-amyrenone (40 mg) and α-amyrin (200 mg). Frs 5–7 proved to be insufficient to purify a compound, while fr. 8 after further CC afforded a mixture of α- and β-amyrin (30 mg). Fr. 9 was discarded and frs 10–14 were pooled together for CC to yield uvaol (40 mg), ursolic acid (150 mg) and mangiferonic acid (150 mg). Similarly, fr. 15 after further CC gave benthamic acid (50 mg) and asiatic acid (60 mg) and fr. 16 mangiferonic acid (30 mg). One tenth portion of fr. 20 after CC and TLC (EtOAc–MeOH, 19:1) gave **1** (R_f 0.48, 150 mg) and a complex mixture which after acetylation with Ac_2O in pyridine followed by TLC (hexane–EtOAc, 1:1) yielded **2a** (R_f 0.54, 100 mg). Methylation of COOH groups in **1** and **2a** was done by treating with diazomethane and processing as usual to yield **1b** and **2b**.

3,25-*Epoxy*-1,2,3,11-*tetrahydroxyurs*-12-*en*-28-*oic acid* (**1**). Crystals, mp 316–318°; $[\alpha]_D^{30}$ + 51.8° (MeOH: *c* 0.161). IR ν_{max}^{KBr} cm^{-1}: 3600–3200 (OH, COOH), 3000–2910, 1693 (COOH), 1645 (C=C), 1120, 1050 (C-O-C). EIMS *m/z* (rel. int.): 446 $[M-4H_2O]^+$ (3), 249 (53), 248 $[C_{16}H_{24}O_2]^+$ (100), 235 (19), 219 (24), 203 (95), 191 (45), 149 (80), 133 (95), 119 (40), 105 (30), 55 (55). ^1H NMR and ^{13}C NMR: Tables 1 and 2.

3,25-*Epoxy*-1,2,3-*triacetoxyurs*-12-*en*-28-*oic acid* (**2a**). Viscous mass, $[\alpha]_D^{30}$ + 32.6° ($CHCl_3$: *c* 0.265). IR ν_{max}^{KBr} cm^{-1}: 3600–3200 (OH, COOH), 3000–2900, 1747 (acetates), 1710 (COOH), 1645 (C=C), 1235 (acetate), 1110, 1044 (C-O-C). EIMS *m/z* (rel. int.): 568 $[M-2AcOH]^+$ (6), 508 $[M-3AcOH]^+$ (3), 494 (8), 434 (7), 367 (7), 248 $[C_{16}H_{24}O_2]^+$ (100), 235 (9), 219 (12), 203 (58), 187 (27), 173 (21), 133 (62), 119 (35), 105 (25),

Table 1. ^{1}H NMR spectral data of compounds **1**, **1a**, **1b**, **2a** and **2b** from the resin of *Shorea robusta* (80 MHz)

H	**1** $(CD_3)_2SO$	**1** C_5D_5N	**1a** $CDCl_3$	**1b** $(CD_3)_2CO$	**2a** $CDCl_3$	**2b** $CDCl_3$
CH(Me)	0.78, 0.90, *d* each (6)	0.83, 0.90, *d* each (6)	0.82, 0.94, *d* each (6)	0.73, 0.92, *d* each (6)	0.92, 0.94, *d* each (6)	0.82, 0.90, *d* each (6)
C(Me)	0.54, 0.91, 0.92, 1.04, *s* each	0.95*, three *s*, 1.05, *s*	0.86*, two *s* 1.06*, two *s* —	0.84, 0.94, 1.04, 1.12 *s* each	0.93, 0.99, *s* each, 1.15*, two *s*	0.76, 0.85 *s* each 1.06*, two *s*
1α	4.01, *d* (4.0)	4.15*	4.96, *d* (4.0)	4.20*	5.06, *d* (4.0)	5.03, *d* (4.0)
2α	4.12, *d* (4.0)	4.15*	5.06, *d* (4.0)	4.20*	5.10, *d* (4.0)	5.10, *d* (4.0)
11β	4.30, *d* (3.5)	4.15*	5.20, *d* (3.5)	4.20*	—	—
12	5.10, *d* (3.5)	5.40, *d* (3.50)	5.27, *d* (3.5)	5.20, *d* (3.5)	5.20, *t* (7.0)	5.20, *t* (7.0)
18	2.45, *d* (10.0)	2.51, *d* (10.0)	†	2.20, *d* (10.0)	†	2.32, *d br* (10.0)
25a	3.40, *d* (11.0)	3.58, *d* (11.0)	3.52, *d* (11.0)	3.48, *d* (11.0)	3.55, *d* (11.0)	3.80, *d* (11.0)
25b	4.06, *d* (11.0)	4.06, *d* (11.0)	3.82, *d* (11.0)	3.90, *d* (11.0)	3.85, *d* (11.0)	3.48, *d* (11.0)
OAc	—	—	1.95, 1.99, 2.06, 2.18, *s* each	—	1.95, 1.98, 2.04, *s* each	1.94, 1.98, 2.04, *s* each
CO_2Me	—	—	—	3.45, *s*	—	3.50, *s*

J values in Hz in parentheses.
* Overlapping signals.
† Signals obscured.

Table 2. ^{13}C NMR spectral data of compounds **1a** and **2a** from the resin of *Shorea robusta* (100 MHz, $CDCl_3$)

C	**1a**	**2a**	C	**1a**	**2a**
1	74.9[a]	74.2[a]	20	39.8[b]	39.8[b]
2	75.0[a]	75.6[a]	21	30.2[d]	30.0[d]
3	101.2	100.9	22	36.0	35.8
4	39.8[b]	39.8[b]	23	28.0	28.2
5	52.7[c]	52.7[c]	24	17.1[e]	17.2[e]
6	17.9	18.2	25	65.4	65.9
7	31.0	31.2	26	17.1[e]	17.4[e]
8	42.1	32.0	27	26.0	26.1
9	38.0	38.0	28	182.0	181.6
10	38.0[b]	38.2[b]	29	17.2[e]	17.4[e]
11	69.9[a]	23.5[a]	30	23.8	23.6
12	127.0	125.2	OAc	170.08	170.0
13	138.4	140.1		170.10	170.1
14	42.3	42.1		170.20	170.2
15	19.5[d]	29.3[d]		170.20	20.4
16	23.8	24.0		20.2	20.6
17	47.8	47.2		20.8	21.0
18	62.7[c]	52.2[c]		21.0	
19	44.0	44.0		21.1	

[a–e] Assignments bearing the same superscript may be interchanged with the same column.

69 (25), 55 (36). ^{1}H NMR and ^{13}C NMR: Tables 1 and 2.

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