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PLECTRANTHOIC ACID *A* & *B*, TWO NEW TRITERPENOIDS FROM *PLECTRANTHUS RUGOSUS*

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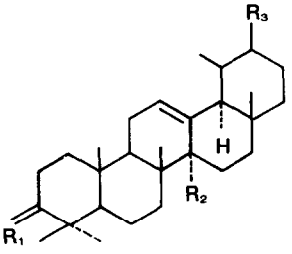
Abstract—Two new triterpenoid acids, designated as plectranthoic acid *A* and plectranthoic acid *B*, have been isolated from *P. rugosus* and are characterised as (20 *S*)-3α-hydroxy-18α,19αH-urs-12-en-30β-oic acid and (14 *S*)-3α-hydroxy-18α, 19α H-urs-12-en-27α-oic acid.

Earlier investigations[1] have revealed the presence of triterpenoids in *P. rugosus*. In this paper we report the isolation and characterisation of two new triterpenoid acids, belonging to the rarely found ψ-taraxastane group of compounds, from the fresh leaves of the title plant.

RESULTS AND DISCUSSION

The methanolic extract of defatted leaves of *P. rugosus*, on chromatographic fractionation, afforded two colourless substances, plectranthoic acid *A*(I) and plectranthoic acid *B*(II), having identical molecular formula $C_{30}H_{48}O_3$. The compounds responded positively to Liebermann–Burchard, TCA and TMN tests. Their IR spectra showed absorptions due to hydroxyl, carboxyl, trisubstituted double bond and gem-dimethyl groups. This suggested that both these compounds are pentacyclic unsaturated triterpenoid acids.



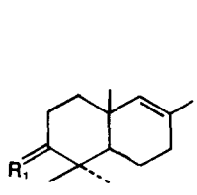
	R_1	R_2	R_3
I.	OH, H	CH_3	COOH
Ia.	OAc, H	CH_3	COOH
Ib.	=O	CH_3	COOH
Ic.	OAc, H	CH_3	$COOCH_3$
II.	OH, H	COOH	CH_3
IIa.	OAc, H	COOH	CH_3
IIb.	=O	COOH	CH_3
IIc.	OAc, H	$COOCH_3$	CH_3

The ^{1}H-NMR of I contained resonance signals attributable to six tert.Me's at δ0.68–0.96, a sec.Me at δ1.05, a carbinylic proton, possibly at the usual C-3 position, at δ4.37 (m, $W_{1/2}$ 16.8 Hz) and a vinylic proton at δ5.12 (br, s). I resisted acetylation with Ac_2O–C_5H_5N at room temperature. However, the compound formed monoacetate **Ia**, $C_{32}H_{50}O_4$, on heating the reaction mixture on water bath for 5.5 h. The ^{1}H-NMR spectrum of **Ia** displayed a resonance signal at δ2.06 due to –OCOCH$_3$; the C-3H was shifted downfield to δ4.60. Sarett oxidation of I readily gave keto acid **Ib**, $C_{30}H_{46}O_3$. The ^{1}H-NMR of **Ib** contained a two proton multiplet at δ2.35 ascribed to C-2 methylene protons. The downfield position of C-3H in I, its multiplicity, coupling constant, ease of oxidation and resistance to acetylation established the configuration of C-3 OH as α- and axial.

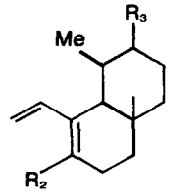
The compound **Ia** reacted with CH_2N_2 to give monomethyl ester **Ic**, $C_{33}H_{52}O_4$. Its ^{1}H-NMR displayed resonance signals due to –COOCH$_3$ and –OCOCH$_3$ at δ3.42 and 2.12, respectively.

The high resolution MS of I signified that double bond triggers the typical retro-Diels–Alder fragmentation[2] of ring C, resulting in a base peak fragment *X* at *m/e* 248 ($C_{16}H_{24}O_2$); the other fragment *Y* is observed at *m/e* 208 ($C_{14}H_{24}O$). I also showed prominent peaks at *m/e* 207 and 189.

The presence of only seven methyls (one secondary) in I and its derivatives coupled with high resolution MS indicated that I had either urs-12-ene or its rearranged skeleton, with one of C-29 or C-30 Me's transformed into –COOH. The chemical shifts of tertiary Me's of I were not in agreement with urs-12-ene skeleton.[3] Further the



Y



X

R_1	m/e at	R_2	R_3	m/e at
OH, H	208	CH_3	COOH	248
OAc, H	250	COOH	CH_3	248
O	206	$COOCH_3$	CH_3	262
		CH_3	$COOCH_3$	262

chemical shift (δ 2.10) and multiplicity (d, J = 5.2 Hz) of C-18 H, in the ^{1}H-NMR of **I-Ib**, indicated that C-18H is α oriented and couples with C-19αH. This established that I has 18α, 19α H-urs-12-ene (19 epi-ψ-taraxastane)[4] skeleton.

The ^{1}H-NMR of **I–Ib** confirmed the secondary nature of the –COOH group; the proton on the carbon carrying the carboxyl group appeared at δ 2.90 (1H, t, J = 16.9, 4 Hz). Since I failed to form a bromo-γ-lactone, on treatment with Br_2–MeOH, expected from C-19β COOH, the carboxyl group must be located at C-20. The exact configuration of C-20 COOH was deduced from the multiplicity and coupling constant of C-20 H together with the study of models. The large coupling constant,

J = 16.9 Hz, indicated that C-20 H undergoes trans diaxial coupling with one of C-21 methylene protons; hence –COOH must be β- and equatorial. Plectranthoic acid *A* is, therefore, (20 *S*)-3α-hydroxy-18α, 19α H-urs-12-en-30β-oic acid.

The ^{1}H-NMR of **II**, revealed the presence of five tertiary Me's at δ 0.77–0.99, two secondary Me's at δ 1.03, a carbinylic proton at δ 4.50 (t, J = 9, 6 Hz), a vinylic proton at δ 5.00 and an allylic proton (C-18 H) at δ 2.90 (d, J = 5 Hz). **II** formed monoacetate **IIa**, $C_{32}H_{50}O_4$, on heating with Ac_2O–C_5H_5N. The ^{1}H-NMR of the acetate contained resonance signal due to –OCOCH$_3$ at δ 2.09 and C-3 H at δ 4.64. The Sarett oxidation of **II** readily yielded ketoacid **IIb**, $C_{30}H_{46}O_3$. This confirmed the presence of C-3αOH in **II**. The monoacetyl derivative formed monomethyl ester **IIC**, on treatment with CH_2N_2; the MS and IR spectrum of **IIc** resembled the spectrum of **Ic**, very closely.

Information about the position of functional groups was gathered from the high resolution MS of **II**. The base peak fragment at *m/e* 248 ($C_{16}H_{24}O_2$) and other prominent fragments at *m/e* 208 ($C_{14}H_{24}O$), 207 ($C_{14}H_{23}O$) and 189 indicated the presence of C-3 OH, C-12 double bond and –COOH in ring D or E. The presence of only five tertiary Me's and two secondary Me's in the parent compound suggested that it contained –COOH at either C-14 or C-17. The absence of –COOH at C-17 was indicated by the MS of **II-IIe**; it revealed prominent peaks at *m/e* 441, 483, 439, 497 and 233, respectively, arising due to the loss of C-17 Me from the molecular ions and the base peak fragment. The position of –COOH at C-14 was further supported by the less intense peaks at *m/e* 412 and 204, originating from the molecular ion and the base peak fragment by the loss of CO_2. The close resemblance of the MS of **II** with MS of **I** supplemented by the position and multiplicity of C-18 H as also the chemical shift of tertiary Me's established the similarity in the basic skeleton of two acids. Plectranthoic acid *B* was therefore identified as (14 *S*)-3α-hydroxy-18α, 19α H-urs-12-en-27α-oic acid.

EXPERIMENTAL

M.ps are uncorrected. IR was recorded on KBr, ^{1}H-NMR was run at 220, 90, 60 MHz with TMS as internal standard. High resolution MS was recorded on a JEOL OGC-MS instrument.

Extraction and Isolation. Fresh leaves of *P. rugosus* (8 Kg) after soxhleting with pet ether were re-extracted with hot MeOH for 48 hr and the residue left after removal of the solvent was chromatographed over silica gel column. The development of the column with C_6H_6–EtOAc (9:1) afforded a mixture of three compounds which on repeated chromatography gave **I** (0.290 g). Further development of the column with C_6H_6–EtOAc (1:9) afforded colourless **II** (0.100 g) which was crystallised from Me_2CO–MeOH.

Identification of **I** & **II**. **I**, m.p. 260°, $[\alpha]_D^{25}$ + 59° (c 0.1, MeOH), M^+ at *m/e* 456.3628 (cacl. for $C_{30}H_{48}O_3$, 456.3626). IR: ν_{max} cm^{-1} 3425 (–OH), 2930, 3200–2500 (br, –COOH), 1695, 1680, 1380, 1360, 1020, 820. ^{1}H-NMR (220 MHz), δ 0.68 (3H, s, C-24 Me), 0.72 (3H, s, C-25 Me), 0.80–0.90 (9H, s, C-23, C-26, C-27 Me's), 0.96 (3H, s, C-28 Me), 1.05 (3H, C-29 Me), 1.30–1.80 (18H, m), 2.10 (1H, d, J = 5.2 Hz), 2.90 (1H, t, J = 16.9, 4 Hz), 4.37 (1H, m, *W*1/2 16.8 Hz), 5.12 (1H, s, br). MS: 456 (M^+), 438 (M^+–H_2O), 411, 367, 366, 248 (100%), 208, 207, 204, 203, 189.

II, m.p. 307–308°, $[\alpha]_D^{25}$ + 62° (c 0.12, MeOH), M^+ at *m/e* 456.3607, $C_{30}H_{48}O_3$. Its IR resembled **I** very closely. ^{1}H-NMR (90 MHz), δ 0.69 (3H, s, C-24 Me), 0.70 (3H, s, C-25 Me), 0.85 (9H, s, C-23, C-26, C-28 Me's), 1.03 (6H, s, C-29, C-30 Me's), 2.90 (1H, d, J = 5 Hz, C-18 H), 4.50 (1H, t, J = 9, 6 Hz), 5.0 (1H, br, s). MS: 456 (M^+), 441 (M^+ – Me), 4.39, 438 ($M^+ - H_2O$), 423 ($M^+ - H_2O$–Me), 412 (M^+–CO_2), 410, 408, 395, 300, 287, 257, 256, 248 (100%), 233, 219, 207, 205, 189.

Acetylation of **I** & **II**. **I** (150 mg) and **II** (90 mg) in C_5H_5N (1 ml) were heated with AC_2O (4 & 2 ml) on water bath for 5.5 and 5.0 h, respectively. After usual working **Ia** (120 mg) and **IIa** (60 mg) were recovered. **Ia**, m.p. 245°, M^+ at *m/e* 498.0188; **IIa**, m.p. 250°, M^+ at *m/e* 498.0176 (calc. for $C_{32}H_{50}O_4$, 498.0188). IR: ν_{max} cm^{-1} 2920, 3200–2500 (br), 1735, 1680, 1380, 1360, 1245 and 820. ^{1}H-NMR: (60 MHz), $CDCl_3$; **Ia** δ 0.70–0.95 (18H, 6 × tert. Me's), 1.08 (3H, s, C-29 Me), 2.06 (3H, s), 2.10 (1H, d, J = 6 Hz, C-18H), 2.90 (1H, t, J = 16.9, 4 Hz), 4.60 (1H, m, W1/2 16.5 Hz), 5.12 (1H, br, s). **IIa** δ 0.67–0.82 (15H, 5 × tert. Me's), 1.10 (6H, 2 × sec. Me's), 2.14 (1H, d, J = 5.5 Hz), 2.30 (3H, s), 4.64 (1H, t, J = 8, 6.5 Hz), 5.0 (1H, s, br). MS: 498 (M^+), 438, 423, 300, 250, 248 (100%), 205, 203, 189.

Sarett oxidation of **I** & **II**. The soln of **I** (18 mg) & **II** (20 mg) in C_5H_5N (1 ml) were added to a well stirred ice cold suspension of CrO_3–C_5H_5N (from 6 ml of C_5H_5N and 0.6 g of CrO_3). The mixtures were diluted with 0.01% HCl (40 ml) after 5 h and extracted with ether. After removal of the solvent, the residues were purified by crystallisation from C_6H_6-pet. ether to give **Ib** and **IIb**. M^+ at *m/e* 454.3440 (**Ib**), 454.3420 (**IIb**), (cacl. for $C_{30}H_{46}O_3$, 454.3447). IR: ν_{max} cm^{-1} 2930, 3200–2500 (br), 1705, 1680, 820. ^{1}H-NMR: (60 MHz), $CDCl_3$; **Ib** δ 0.70–0.92 (6 × tert. Me's), 1.06 (3H, sec. Me), 2.35 (2H, m, C-2 H's), 2.85 (1H, t, J = 16.9, 4.2 Hz), 5.16 (1H, br, s). **IIb** 0.70–0.85 (5 × tert. Me's), 1.10 (6H, 2 × sec. Me's), 2.30 (2H, m), 5.02 (1H, br, s). MS: 454 (M^+), 439, 396, 380, 355, 248 (100%), 206, 203, 189.

Methylation of **Ia** & **IIa**. The solns of **Ia** (60 mg) and **IIa** (45 mg) in ether were treated with freshly prepared CH_2N_2. After usual work up **Ic** (50 mg), m.p. 197°, and **IIc** (35 mg), m.p. 254°, were obtained. M^+ at *m/e* 512.3862 (cacl. for $C_{33}H_{52}O_4$, 512.3865). IR: ν_{max} cm^{-1} 2935, 1735, 1720, 1245, 1380, 1360, 820. ^{1}H-NMR: (60 MHz), $CDCl_3$; **Ic** δ 0.68–1.10 (6 × tert. Me's), 1.09 (3H, sec. Me), 2.12 (3H, s), 2.20 (1H, d, J = 5 Hz), 2.80 (1H, t, J = 16.5, 4 Hz), 3.42 (3H, s), 4.55 (1H, m, W1/2 16.2 Hz), 5.18 (1H, br, s). **IIc** δ 0.66–0.85 (5 × tert. Me's), 1.12 (6H, sec. Me's), 2.10 (1H, d, J = 6 Hz), 2.22 (3H, s), 3.83 (3H, s), 4.44 (1H, t, J = 9.6 Hz), 5.0 (1H, br, s). MS: 512 (M^+), 497, 482, 420, 262 (100%), 250, 247, 232, 191, 189.

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