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THREE BRIDGED 14β,26-EPOXY-C-HOMO-PENTACYCLIC TRITERPENES FROM *PRIMULA ROSEA**

K. K. BHUTANI,† R. KAPOOR and C. K. ATAL

Regional Research Laboratory (CSIR), Jammu Tawi 180001, India

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Key Word Index—*Primula rosea*; Primulaceae; bridged-14β,26-epoxyserratane (C-homo-pentacyclic) triterpenoids.

Abstract—Three new bridged 14β,26-epoxy-C-homo-pentacyclic triterpenes isolated from *Primula rosea* have been shown to be 14β,26-epoxy-serratane-3,21-dione, 21α-hydroxy-14β,26-epoxy-serratane-3-one and 21β-hydroxy-14β,26-epoxy-serratane-3-one, respectively, on the basis of ^{1}H NMR, ^{13}C NMR and mass spectral and chemical evidence.

INTRODUCTION

Rosinidin has been isolated from *Primula rosea* [1]. However, no report is available on the triterpene constituents of this plant. The plant materials for the present study were collected from Gulmarg (Kashmir) in July. The isolation of three novel triterpenes having a bridged epoxy-linkage from this plant and the spectral and chemical evidence leading to the elucidation of their structures and stereochemistries are discussed in this paper.

RESULTS AND DISCUSSION

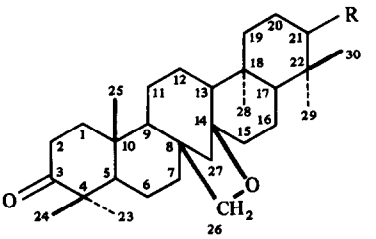
The petrol extract of the whole plant was submitted to column chromatography over neutral alumina to yield compound A (250 mg, 0.25% yield), compound B (200 mg, 0.2% yield) and compound C (250 mg, 0.25% yield).

Compound A had mp 244–246° $[M]^+$ at *m/z* 454 ($C_{30}H_{46}O_3$) and ν_{max}^{KBr} cm^{-1}: 1705 (C=O), 1700 (C=O). Its ^{1}H NMR spectrum showed the presence of six tertiary methyl groups on saturated carbons [δ 0.88 (3H, *s*), 0.97 (3H, *s*), 1.03 (6H, *br s*), 1.32 (6H, *s*)], two *gem.*-coupled protons at 2.58 (1H, *d*, *J* = 14 Hz) and 2.72 (1H, *d*, *J* = 14 Hz) and two –CH_2–O– coupled protons at 3.44 (1H, *d*, *J* = 8 Hz) and 3.92 (1H, *d*, *J* = 8 Hz). The ^{13}C NMR spectrum further showed the presence of six tertiary methyl carbons at δ 15.8, 18.2, 21.2, 21.6, 26.7, 33.1; two deshielded methylene carbons at 49.7 and 75.3; one deshielded quaternary carbon at 86.4 and two carbonyl carbons at 213.8 and 218.1.

These data suggested the presence of the serratane skeleton and the fact that only six tertiary methyl signals were present in the ^{1}H NMR and ^{13}C NMR spectra showed that one methyl was involved in the formation of the C-homo ring. The presence of two carbonyls was indicated by the IR and ^{13}C NMR spectra and suggested that the third oxygen atom may be in the form of an epoxy-linkage. Two A/B type coupled methylene protons resonating at δ 3.44 and 3.92 in the ^{1}H NMR spectrum were involved in the ether bridge attached to the main pentacyclic skeleton. Since onocerane is the precursor of serratane [2], the C-26 methyl group could be assumed to form a bridge through the 14β-position. The presence of signals for two *gem.*-coupled methylene protons at δ 2.58 and 2.72 (due to the electronegative effect of bridged oxygen) in the ^{1}H NMR spectrum of compound A could thus be assigned to the C-27 methylene of the C-homo ring.

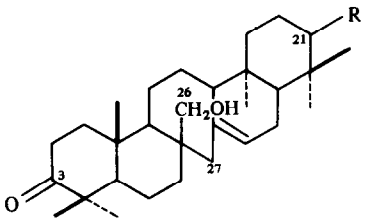
The epoxy-linkage was opened by the addition of a drop of Lewis acid (BF_3–etherate) to give the diketoenol **4**, mp 275–276°; ν_{max}^{KBr} cm^{-1}: 3400, 2930, 2845, 1705, 1700, 1440, 1378, 1115 and 980; ^{1}H NMR ($CDCl_3$): δ 0.78 (3H, *s*), 0.94 (3H, *s*), 0.98 (3H, *s*), 1.0 (3H, *s*), 1.02 (3H, *s*), 1.36 (3H, *s*) (six tertiary methyls on saturated carbons); 1.7 (1H, *br s*, D_2O exchangeable); 3.05–3.32 (2H, *m*, –CH_2–OH) and 5.48 (1H, *m*, H-15). The diketoenol **4** underwent acetylation with Ac_2O–pyridine to give a monoacetylated product (**7**), mp 248–250°; ν_{max}^{KBr} cm^{-1}: 2960, 1735, 1700 and 1240; ^{1}H NMR ($CDCl_3$): δ 0.78 (3H, *s*), 0.94 (3H, *s*), 0.98 (3H, *s*), 1.0 (3H, *s*), 1.02 (3H, *s*), 1.36 (3H, *s*) (six tertiary methyls on saturated carbons); 2.03 (3H, *s*, OCOMe); 3.97–4.30 (2H, *m*, CH_2–OAc) and 5.48 (1H, *m*, H-15) proving that the third oxygen atom was in the form of a cyclic ether. Thus the structure of compound A is 14β,26-epoxy-serratane-3,21-dione (**1**).

The location of the epoxy-linkage was further revealed and confirmed by the general mass fragmentation be-

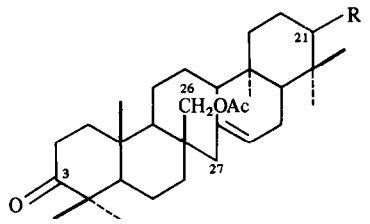


	R
1	O
2	α – OH
3	β – OH

*Part 1 in the series "Investigations of Medicinal Plants".
†To whom correspondence should be addressed.



	R
4	O
5	α – OH
6	β – OH



	R
7	O
8	α –OH
9	β –OAc

haviour of compound **1**. The significant ions with appropriate mass shifts in their mass spectra arise by transfer of a hydrogen atom in the saturated triterpenes [3] during the fragmentation process to give a radical ion (**a**) at *m/z* 248 (80.6%) and an anion(**b**) at *m/z* 205 (68.1%). The ions at *m/z* 217 (66.5%) and 203 (100%) are explained by the loss of 31 amu (CH_2O) and 14 amu (^+Me), respectively, from the fragment at *m/z* 248 (80.6%).

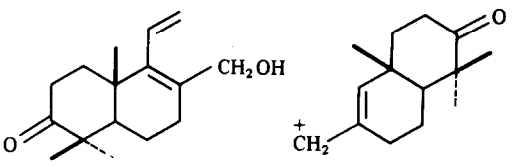
The other important fragment (**c**) at *m/z* 235 (98.6%) was obtained by an alternative transfer of a hydrogen atom. The ion at *m/z* 424 (21.3%) was due to the loss of $-CH_2O$ from the molecular ion peak.

The ion at *m/z* 203 (100%) may result from centrally cleaved fragments from **d** derived by loss from the molecular ion of two angular methyls and a water molecule from the ether bridge through charge transfer.

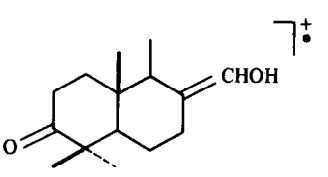
Compound B had mp 215–217°, ν_{max}^{KBr} cm^{-1}: 3420 (–OH), 1700 (C=O), 1110 (C–O stretch of hydroxy); ^1H NMR ($CDCl_3$): six tertiary methyls on saturated carbon atoms (δ 0.84, 0.94, 0.98, 1.01, 1.06, 1.32, 3H, *s* each), 1.67 (1H, *s*, D_2O exchangeable) two *gem.*-coupled protons of C-27 methylene at 2.58 (1H, *d*, *J* = 14 Hz) and 2.72 (1H, *d*, *J* = 14 Hz), a deshielded methine proton on which –OH was attached at δ 3.20 (1 H, *m*), two $-CH_2-O-$ coupled protons on C-26 involved in the ether bridge at 3.50 and 3.94 (1H, *d*, *J* = 8 Hz, each); MS *m/z* 456 $[M]^+$ (32%); $C_{30}H_{48}O_3$ calculated from high resolution. Compound C had mp 299–301°, ν_{max}^{KBr} cm^{-1}: 3420 (–OH), 1700 (C=O), 1110 (C–O stretch of hydroxy); ^1H NMR ($CDCl_3$): six tertiary methyls on saturated carbon atoms [δ 0.84, (3H, *s*), 0.97 (6H, *br s*), 1.02 (6H, *br s*), 1.28 (3H, *s*)], 1.70 (1H, *s*, D_2O exchangeable), two *gem.*-coupled protons of the C-27 methylene at 2.58 (1H, *d*, *J* = 14 Hz) and 2.75 (1H, *d*, *J* = 14 Hz), a deshielded methine proton on which –OH was attached at 3.20 (1H, *m*), two $-CH_2-O-$ coupled protons on C-26 involved in the ether bridge at 3.50 and 3.94 (1H, *d*, *J* = 8 Hz, each); MS *m/z* 456 $[M]^+$ (6%), $C_{30}H_{48}O_3$ calculated from high resolution.

Thus compounds B and C are similar to each other and resemble compound **1**. The only differences in compounds B and C are that one of the carbonyl groups in compound **1** is reduced and the position and stereochemistry of the resulting hydroxy group vary between them. Compound B resisted acetylation with Ac_2O–pyridine at room temperature whereas compound C gave a monoacetylated product. Compounds B and C were treated with BF_3–etherate and acetylated to give monoacetylated (**8**) and diacetylated (**9**) products. In the mass spectrum of **8** a peak at *m/z* 465 $[M-33]^+$ [4] and other almost similar fragments to those in the mass spectrum of compound **1** proved that the hydroxy group in compound B was in the axial position at C-21 and thus led to the assigned structure **2**. Similarly, the structure of compound C was assigned as **3** on the basis of similar mass spectral fragments obtained for **3** compared to compound **1**. The hydroxy group is placed at C-21 in the equatorial position because of its easy accessibility for acetylation.

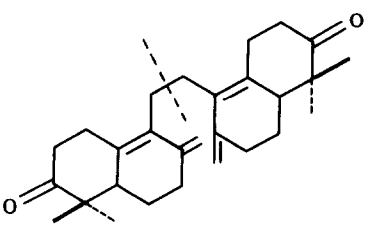
Finally, a detailed comparative study of the mass spectral fragmentation patterns of all the above compounds was made, and in general, it was concluded that the fragmentations were in good agreement with a saturated serratane skeleton. The fragmentation pattern of the 14-serratene skeleton is well established [5–7]. The ^{13}C NMR spectra of serratanes have not been reported in the literature. The ^{13}C NMR spectra (Table 1) established



a **b**



c



d

Table 1. ^{13}C NMR chemical shifts of compounds **1**, **2** and **3** in $CDCl_3$

	Carbon									
	1	2	3	4	5	6	7	8	9	10
1	39.5	34.2	218.1	47.7	55.2	19.0	33.5	56.3	39.9	36.9
2	38.9	33.7	213.8	49.7	55.4	17.7	33.2	56.3	40.1	37.1
3	38.9	33.7	213.8	49.7	55.4	17.7	33.2	56.3	40.1	37.1

	Carbon									
	11	12	13	14	15	16	17	18	19	20
1	19.0	24.8	54.7	86.4	45.7	23.6	29.8	49.9	35.5	31.9
2	18.6	24.9	54.7	86.4	45.6	23.6	29.8	50.5	35.2	31.6
3	18.6	24.9	54.7	86.4	45.6	23.6	29.8	50.5	35.2	31.6

	Carbon									
	21	22	23	24	25	26	27	28	29	30
1	213.8	42.7	26.7	21.2	15.8	75.3	49.7	18.2	33.1	21.6
2	79.2	42.8	27.4	16.0	15.3	75.4	49.9	28.2	32.3	21.7
3	79.2	42.8	27.4	16.0	15.3	75.4	49.9	28.2	32.3	21.7

that compounds **1**, **2** and **3** were bridged 14β,26-epoxy-serratanes. The signal assignments for the carbons of rings A and B were deduced by comparison with the chemical shifts reported for the α- and β-amyrins and other pentacyclic triterpenoids of the oleanene series [8–12]. The assignments of the signals for rings C, D and E carbons were based on the chemical shift rules [13, 14] and a study of Dreiding Models.

The C-26 signal (involved in the ether linkage) was downfield at δ 75.3–75.4 in compounds **1–3** and is comparable with the CH_2–O signal reported for saikogenins [9, 10]. As expected from the Drieding model, the signal for C-27 was deshielded at 49.7–49.9. Deshielded quaternary carbon signals at 56.3 and 86.4 have been assigned to C-8 and C-14 in comparison to similar signals reported for saikogenins [9, 10].

EXPERIMENTAL

Mps are uncorr. ^1H NMR at 60 MHz; ^{13}C FT-NMR were recorded on a Jeol model FX-90 θ at 22.49 MHz in $CDCl_3$ with TMS as an int. standard (δ C, O) in 5 mm spinning tubes at room temp. Concns of compounds were *ca* 0.2–0.5 mmol/ml. FT-measurement conditions were: spectral width 5648 Hz; pulse flipping angle *ca* 45°; acquisition time 0.75 sec; number of data points 10000. Column chromatography (CC) was carried out using neutral Al_2O_3; TLC was on silica gel, solvent systems hexane–EtOAc (4:1) and (2:1), C_6H_6–Me_2CO (4:1); cerric ammonium sulphate–H_2SO_4 soln was used as spraying reagent for TLC plates.

Extraction. Dried and powdered whole plant (800 g) of *Primula rosea* was extracted exhaustively in a Soxhlet apparatus with petrol. The petrol extract (10 g) was chromatographed on a neutral Al_2O_3 column (300 g) using solvents and solvent mixtures of increasing polarity. Similar fractions as indicated by TLC were combined.

Isolation of n-*triancontane and* n-*triancontinol.* The petrol eluate on crystallization from petrol–Me_2CO afforded white flakes (1.0 g), mp 63–64°. It proved identical with triancontane on TLC (hexane–EtOAc, 4:1).

The later fractions eluted with petrol afforded 900 mg of a crystalline compound, mp 71–72°, crystallised from petrol–Me_2CO identical with *n*-triancontinol by TLC, and mmp.

Isolation of 14β,26-*epoxy-serratane*-3,21-*dione* (**1**). The petrol–C_6H_6 (3:2) eluate fractions (8 × 100 ml) from the Al_2O_3 column afforded compound **1** (250 mg; 0.25% yield), mp 244–246° after crystallization from petrol–Me_2CO. IR ν_{max}^{KBr} cm^{-1}: 2930, 2845, 1705, 1700, 1440, 1378, 980. MS *m/z* (rel. int.): 454 $[M]^+$ (52), 424 (21), 248 (81), 235 (99), 217 (66), 205 (68), 203 (100), 191 (15), 123 (41), 109 (33), $C_{30}H_{46}O_3$ calc. from high resolution.

Isolation of 21α-*hydroxy*-14β,26-*epoxy-serratane*-3-*one* (**2**). The later C_6H_6–$CHCl_3$ (4:1) eluate fractions (4 × 100 ml) of the main chromatogram furnished compound **2** which was purified to homogeneity through rechromatography on a neutral Al_2O_3 (50 g) column in $CHCl_3$ and crystallized from MeOH (200 mg, 0.2% yield, mp 215–217°). IR ν_{max}^{KBr} cm^{-1}: 3420, 2865, 1700, 1450, 1345, 1300, 1110, 1020, 980. MS *m/z* (rel. int.): 456 $[M]^+$ (32), 426 (10), 385 (5), 248 (89), 235 (100), 217 (15), 207 (50), 205 (49), 203 (95), $C_{30}H_{48}O_3$ calc. from high resolution.

Isolation of 21β-*hydroxy*-14β,26-*epoxy-serratane*-3-*one* (**3**). Further $CHCl_3$ fractions (4 × 100 ml) of the chromatography furnished compound **3** which was purified on a small neutral Al_2O_3 (50 g) column in $CHCl_3$ (250 mg, 0.25% yield) after crystallization with MeOH, mp 299–301°: IR ν_{max}^{KBr} cm^{-1}: 3420, 2865, 2820, 1700, 1450, 1375, 1300, 1110, 1020, 980. MS *m/z* (rel. int.): 456 $[M]^+$ (6), 426 (2), 248 (18), 235 (21), 217 (12), 207 (10), 205 (10), 203 (24), $C_{30}H_{48}O_3$ calc. from high resolution.

Acetylation of compound **3**. The acetate recrystallized with MeOH as needles, mp 265–266°: IR ν_{max}^{KBr} cm^{-1}: 2865, 1735, 1700, 1250; ^1H NMR: δ 0.84 (3H, *s*), 0.98 (6H, *br s*), 1.02 (6H, *br s*), 1.28 (3H, *s*), 2.03 (3H, *s*), 2.58 (1H, *d*, J = 14 Hz), 2.75 (1H, *d*, J = 14 Hz), 3.50 (1H, *d*, J = 8 Hz), 3.98 (1H, *d*, J = 8 Hz), 4.48 (1H, *m*). $[M]^+$ at *m/z* 498, $C_{32}H_{50}O_4$ calc. from high resolution.

26-*Hydroxymethyl*-14-*serratene*-3,21-*dione* (**4**). Compound **1** (80 mg) in dry Et_2O was treated with 2–3 drops of a freshly

distilled ethereal soln of BF_3 and the mixture was immediately worked up as usual to give 60 mg of a semi-solid which on crystallization from Me_2CO gave product **4**, mp 275–276°; IR ν_{max}^{KBr} cm^{-1}: 3400, 2930, 2845, 1705, 1700, 1440, 1378, 1115, 980; ^1H NMR ($CDCl_3$): δ 0.78, 0.94, 0.98, 1.0, 1.02, 1.36 (3H, *s* each), 1.7 (1H, *br s*, D_2O exchangeable), 3.05–3.32 (2H, *m*), 5.48 (1H, *m*). $[M]^+$ at *m/z* 454, $C_{30}H_{46}O_3$ calc. from high resolution.

26-*Acetoxymethyl*-14-*serratene*-3,21-*dione* (**7**). The diketoenol product (**4**) (50 mg) in pyridine (1.5 ml) was treated with Ac_2O (3 ml, room temp., 8 hr). The residue obtained after usual work-up afforded a gummy mass crystallized from MeOH (50 mg), mp 248–250°; IR ν_{max}^{KBr} cm^{-1}: 2960, 1735, 1700, 1240; ^1H NMR ($CDCl_3$): δ 0.78, 0.94, 0.98, 1.0, 1.02, 1.36, (3H, *s* each), 2.03 (3H, *s*), 3.97–4.30 (2H, *m*), 5.48 (1H, *m*). $[M]^+$ at *m/z* 496, $C_{32}H_{48}O_4$ calc. from high resolution.

21α,26-*Dihydroxy*-14-*serratene*-3-*one* (**5**). Compound **2** (100 mg) in dry Et_2O was treated with 2–3 drops of a freshly distilled ethereal soln of BF_3 and the reaction mixture immediately worked up as usual to obtain a gummy residue (75 mg) which could not be crystallized.

26-*Acetoxy*-21α-*hydroxy*-14-*serratene*-3-*one* (**8**). Compound **5** (75 mg) was dissolved in dry pyridine (1.5 ml) and treated with Ac_2O (3 ml, room temp., 8 hr). The residue obtained after usual work-up of the reaction mixture afforded a gummy mass crystallized from MeOH (50 mg), mp 262–264°; IR ν_{max}^{KBr} cm^{-1}: 3420, 2960, 1735, 1705, 1250; ^1H NMR ($CDCl_3$): δ 0.78, 0.94, 0.98, 1.0, 1.07, 1.36 (3H, *s* each), 1.75 (*s*, 1H, D_2O exchangeable), 3.20 (1H, *m*), 2.03 (3H, *s*), 3.97–4.30 (2H, *m*), 5.48 (1H, *m*); MS *m/z* (rel. int.): 498 $[M]^+$ (5), 483 (1), 465 (1), 438 (4) 424 (2.5), 248 (18.9), 235 (24.9), 207 (2.6), 203 (27.4), 43 (100), $C_{32}H_{50}O_4$ calc. from high resolution.

21β,26-*Dihydroxy*-14-*serratene*-3-*one* (**6**). Compound **3** (100 mg) in dry Et_2O was treated with 2–3 drops of a freshly distilled ethereal soln of BF_3 and the reaction mixture immediately worked up as usual to obtain a gummy residue (80 mg) which could not be crystallized.

21β,26-*Diacetoxy*-14-*serratene*-3-*one* (**9**). Compound **6** (80 mg) was dissolved in dry pyridine (1.5 ml) and treated with Ac_2O (3 ml, room temp., 8 hr). The residue obtained after usual work-up afforded a gummy mass crystallized from MeOH (60 mg), mp 256–258°; IR ν_{max}^{KBr} cm^{-1}: 2960, 1745, 1735, 1700, 1250; ^1H NMR ($CDCl_3$); δ 0.78, 0.94, 0.98, 1.0, 1.07, 1.36 (3H, *s* each), 2.03 (3H, *s*), 2.05 (3H, *s*), 3.97–4.30 (2H, *m*), 4.50 (1H, *m*), 5.48 (1H, *m*). $[M]^+$ at *m/z* 540, $C_{34}H_{52}O_5$ calc. from high resolution.

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