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TRITERPENOIDS FROM *CORNUS CAPITATA**

RAJENDRA S. BHAKUNI, YOGENDRA N. SHUKLA and RAGHUNATH S. THAKUR

Central Institute of Medicinal and Aromatic Plants, Lucknow, 226016, India

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Key Word Index—*Cornus capitata*; Cornaceae; triterpenoids; tirucallane derivatives.

Abstract—The structure of two new triterpenes isolated from the stems and stem bark of *Cornus capitata* has been elucidated as 21,23-epoxytirucalla-7,24-diene-3-one and 3β-acetoxy-21,23-epoxytirucalla-7,24-diene by physicochemical methods.

INTRODUCTION

Leaves of *Cornus capitata* Wall. possess antiviral [1] and semen coagulating properties [2], while the seeds contain alkaloids [3]. Cornin and phlorin have been isolated from the leaves and twigs [4]. Because the stems had not been examined, a detailed study was undertaken. In our earlier communications, we reported on the isolation of 19 known compounds [5] and two new minor triterpenoids, 3α-acetoxy-2β-hydroxylupan-28, 13β-olide and 3β-acetoxy-23-oxo-lup-20(29)-ene [6]. This report describes the characterization of two new tirucallane derivatives.

RESULTS AND DISCUSSION

Compounds **1a** and **1c** were isolated from the stems and stem bark of *C. capitata*. The IR spectrum bands of **1a** corresponded to keto, *gem* dimethyl, epoxy and trisubstituted double bond functions. A molecular ion at *m/z* 438 in the mass spectrum of **1a** together with elemental analysis suggested the molecular formula was $C_{30}H_{46}O_2$.

Reduction of **1a** with $NaBH_4$ furnished an alcohol (**1b**), $[M]^+$ 440 ($C_{30}H_{48}O_2$) in which the carbonyl absorption of **1a** was replaced by a hydroxyl absorption at 3480 cm^{-1}. Acetylation of **1b** yielded a monoacetate (**1c**), $[M]^+$ 482 ($C_{32}H_{50}O_3$). A characteristic ion present in the mass spectrum of **1a** at *m/z* 271 (corresponding ion in **1b** at *m/z* 273) can be rationalized in terms of loss of the side chain (*m/z* 125) with C-15, C-16 and C-17 through internal hydrogen transfer from C-18 [7]. A Euphane or tirucallane skeleton in **1a** was supported by the ^1H NMR spectra of **1b** and **1c** (see Experimental) where, except for vinylic methyl groups at C-25, no methyl groups appeared more downfield than δ0.97 nor upfield of δ0.70. These data indicated that **1a** was a member of either the Δ^7-euphane or Δ^7-tirucallane series [8–10]. The (−)optical rotations of **1a**, **1b** and **1c** indicated that they belonged to the tirucallane rather than euphane series [9]. A strong ion at *m/z* 425 in **1b** formed by the loss of a methyl at C-14 (corresponding ions as base peaks at *m/z* 423 and 467 in **1a** and **1c**, respectively) also suggested the assignment of one double bond at C-7 rather than at C-9(11) since an allylic cation results from this process [11].

The ^1H NMR spectrum of **1a** displayed five tertiary methyl groups between δ0.80 and 1.06, two vinylic methyl groups at δ1.67, a triplet, J = 9 Hz at δ3.27 and a double doublet, J = 11 and 3 Hz at δ3.96 for C-21 methylene protons, and a multiplet at δ4.60 for a C-23 methine proton. The chemical shifts of H-21 and H-23 suggested the attachment of an epoxy function at these positions forming a tetrahydrofuran ring in the side chain. Further, H-23 was in the more downfield region than H-21 indicating a second double bond at C-24 (δ5.12). The ions at *m/z* 125 (side chain, $C_8H_{13}O$), 313 [M − side chain]$^+$ and others at *m/z* 83 $[Me_2\text{–}C{=}CH{\equiv}O]^+$, 70 $[C_4H_6O]^+$ and 68 $[C_4H_4O]^+$ also showed the presence of tetrahydrofuran ring in the side chain. The Δ^7-double bond resonated as a multiplet at δ5.26 and the C-3 methine proton in **1b** and **1c** appeared at δ3.20 and 4.48, respectively as a triplet (J = 8Hz). Biogenetically, one hydroxyl group could be placed at C-3 and from the J values it had a β-configuration. Thus the keto group in **1a** was assigned at C-3.

Mercuric acetate oxidation of **1a** provided a conjugated diene (**2**) $[M]^+$ 436($C_{30}H_{44}O_2$), which had λ_{max} 230, 237 and 246 nm characteristic of a 7,9(11)-hetero-annular diene in this skeleton [12].

Hydrogenation of **1a** with palladium on activated charcoal yielded two products, **3** and **4a**. The major product (**3**), $[M]^+$ 440($C_{30}H_{48}O_2$), had an ion at *m/z* 127 showing that the side chain double bond was reduced. The C-24 olefinic proton appearing at δ5.12 in **1a** was no longer seen but a C-7 double bond was still present (δ5.30). The C-23 proton and C-26, 27 methyl groups were shifted upfield to δ2.80 and δ0.85, respectively.

The minor hydrogenated product (**4a**), $[M]^+$ 442 ($C_{30}H_{50}O_2$), formed a monoacetate (**4b**), $[M]^+$ 484($C_{32}H_{52}O_3$). The ion at *m/z* 129 (corresponding ion in **4b** at *m/z* 171) indicated that hydrogenation of the Δ^{24}-double bond was accompanied by opening of the epoxy ring. The successive loss of side chain was seen at *m/z* 43, 57, 71 and 85. ^1H NMR signals corresponding to vinylic methyl groups, the Δ^{24}-double bond and the tetrahydrofuran ring of the side chain were absent, instead, C-26 and C-27 methyl groups appeared at δ0.86

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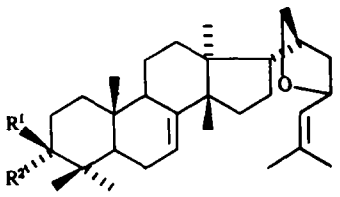
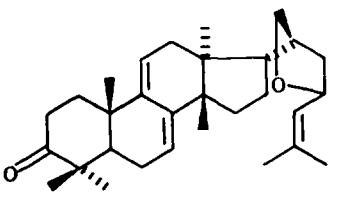


1a $R^1 = R^2 = O$
1b $R^1 = OH$, $R^2 = H$
1c $R^1 = OAc$, $R^2 = H$



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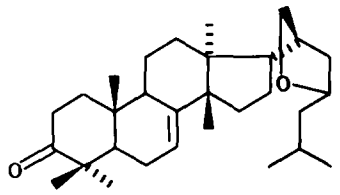

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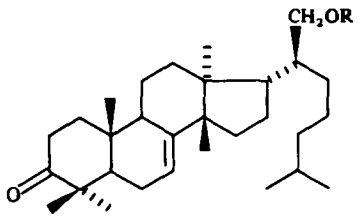
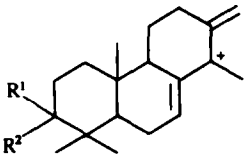
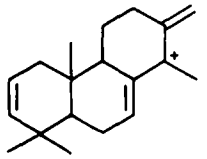


4a R = H
4b R = Ac



a $R^1 = R^2 = O$, *m/z* 271
b $R^1 = OH$, $R^2 = H$, *m/z* 273



c *m/z* 255

(*d*, J = 6 Hz) and the C-21 methylene protons of the newly formed CH_2OH group appeared at δ3.65 (*m*, $W_{1/2}$ = 16 Hz).

These data were fully consistent with the structure of **1a** as 21,23-epoxytirucalla-7,24-diene-3-one. It was further substantiated by its ^{13}C NMR spectrum (Table 1) which was in complete agreement with those recorded for Δ^7-tirucallanes [13, 14].

Compound **1c** was characterized as 3β-acetoxy-21,23-epoxytirucalla-7,24-diene by comparison of its $[\alpha]_D$, mp, and spectral data with those of **1c** obtained from **1a** and by its conversion into **1b**.

Table 1. ^{13}C NMR data for compound **1a** (100 MHz, δ,$CDCl_3$)

C		C	
1	38.5 *t*	16	27.5 *t*
2	34.6 *t*	17	44.0 *d*
3	207.3 *s*	18	12.7 *q*
4	47.8 *s*	19	21.5 *q*
5	52.5 *d*	20	37.4 *d*
6	24.3 *t*	21	73.5 *t*
7	118.0 *d*	22	35.2 *t*
8	145.7 *s*	23	75.5 *d*
9	48.5 *d*	24	127.0 *d*
10	34.8 *s*	25	135.0 *s*
11	18.1 *t*	26	25.7 *q*
12	32.5 *t*	27	17.8 *q*
13	42.2 *s*	28	27.4 *q*
14	50.8 *s*	29	24.5 *q*
15	34.5 *t*	30	22.6 *q*

EXPERIMENTAL

Mps: uncorr; IR: KBr; UV: MeOH; ^1H NMR: 80 MHz, $CDCl_3$ with TMS as int. standard. TLC: silica gel G, spots visualized by exposure to I_2 vapour. The homogeneity of the isolates was checked on TLC in at least three different solvent systems. Plant material was collected from Ranikhet, Uttar Pradesh, and identified in our Botany Department where a voucher specimen has been deposited.

Isolation. Dried and powdered stems and stem bark of *C. capitata* Wall. (3 kg) were extracted with EtOH (9 × 10 l) and the extract was concd *in vacuo* to 200 ml. After the addition of H_2O (200 ml), the extract was extracted with *n*-hexane (7 × 400 ml), $CHCl_3$ (6 × 400 ml), and *n*-BuOH (4 × 400 ml). Removal of solvent from the hexane extract provided a residue (26 g), a

portion (24 g) of which was chromatographed over silica gel (970 g, 60–120 mesh, BDH), eluting with hexane, hexane–C_6H_6 (3:1, 1:1, 1:3), C_6H_6, C_6H_6–$CHCl_3$ (3:1, 1:1, 1:3), $CHCl_3$, $CHCl_3$–MeOH (99:1, 95:5, 90:10) and MeOH. Fractions each of 250 ml were collected and monitored by TLC.

21,23-*Epoxytirucalla*-7,24-*diene*-3-*one* (**1a**). Removal of solvent from fractions 223–252 of the hexane–C_6H_6 (1:3) eluates gave a residue, mp 188° (hexane), 195 mg, $[\alpha]_D$ – 24° ($CHCl_3$). IR ν_{max} cm^{-1}: 2940, 2850, 1700, 1640, 1445, 1384, 1372, 1255, 1158, 1058, 1040, 930, 838, 822; ^1H NMR: δ0.80 (3H, *s*, 18-H_3), 1.06 (3H, *s*, 19-H_3), 0.98 (6H, *s*, 28-H_3, 29-H_3), 1.02 (3H, *s*, 30-H_3), 1.67 (6H, *s*, 26-H_3, 27-H_3), 1.90 (1H, *m*, 20-H), 2.10 (2H, *m*, 22-H_2), 2.28 (2H, *t*, J = 8 Hz, 2-H_2), 3.27 (1H, *t*, J = 9 Hz, 21-H), 3.96 (1H, *dd*, J = 11, 3Hz, 21-H), 4.60 (1H, *m*, 23-H), 5.12 (1H, *m*, 24-H), 5.26 (1H, *m*, 7-H); MS *m/z* (rel. int.): 438 $[M]^+$ ($C_{30}H_{46}O_2$) (32), 423 $[M-Me]^+$ (100), 383 $[M-55]^+$ (4), 313 [M – side chain]$^+$ (2), 285(6), 271(a, 3), 189(5), 187(8), 125[side chain]$^+$ 26), 83(42), 81(29), 70(23), 69(54), 68(8), 55(70). (Found: C, 81.85; H, 10.50 %. $C_{30}H_{46}O_2$ requires: C, 82.19; H, 10.60 %).

$NaBH_4$ *reduction of* **1a**. Compound **1a** (40 mg) was dissolved in MeOH (20 ml), $NaBH_4$ (5 mg) added gradually, and the mixture stirred for 3 hr at room temp. The reaction mixture was then diluted with H_2O (50 ml) and extracted with Et_2O (4 × 50 ml). The Et_2O phases were bulked, washed with H_2O (2 × 50 ml) and dried (Na_2SO_4). Removal of the Et_2O furnished **1b**, mp 144–146° (MeOH), 34mg, $[\alpha]_D$-8° ($CHCl_3$). IR ν_{max} cm^{-1}: 3480, 2960, 1640, 1380, 1360, 1260, 1030, 935, 870, 825; ^1H NMR: δ0.72 (3H, *s*, 19-H_3), 0.80 (3H, *s*, 18-H_3), 0.82 (3H, *s*, 29-H_3), 0.94 (6H, *s*, 28-H_3, 30-H_3), 1.66 (6H, *s*, 26-H_3, 27-H_3), 1.92 (1H, *m*, 20-H), 2.08 (2H, *m*, 22-H_2), 3.20 (1H, *t*, J = 8 Hz, 3α-H), 3.33 (1H, *t*, J = 9 Hz, 21-H), 3.94 (1H, *dd*, J = 11, 3 Hz, 21-H), 4.58 (1H, *m*, 23-H) 5.10 (1H, *m*, 24-H), 5.20 (1H, *m*, 7-H); MS *m/z* (rel. int.): 440 $[M]^+$ ($C_{30}H_{48}O_2$) (23), 425 $[M-Me]^+$ (57), 422 $[M-H_2O]^+$ (25), 407 $[M-Me-H_2O]^+$ (6), 385 $[M-55]^+$ (4), 315 [M – side chain]$^+$ (1), 285 (7), 273**b** (2), 255c(8), 189(2), 187(3), 125(sc, 14), 83(13), 81(7), 70(15), 69(11), 68(2), 55(100).

Acetylation of **1b**. Alcohol **1b** (20 mg) was acetylated (C_5H_5N–Ac_2O, 0.5 ml each) overnight at room temp. The usual work-up yielded **1c**, mp 176° (MeOH), 16 mg, $[\alpha]_D$-6° ($CHCl_3$). IR ν_{max} cm^{-1}: 2930, 2850, 1730, 1640, 1440, 1375, 1360, 1242, 1230, 1020, 935, 825; ^1H NMR: δ0.76 (3H, *s*, 19-H_3), 0.82 (3H, *s*, 18-H_3), 0.92 (3H, *s*, 29-H_3), 0.95 (6H, *s*, 28-H_3, 30-H_3), 1.66 (6H, *s*, 26-H_3, 27-H_3), 2.02 (3H, *s*, -OAc), 3.24 (1H, *t*, J = 9 Hz, 21-H), 3.96 (1H, *dd*, J = 11, 3 Hz, 21-H), 4.48 (1H, *t*, J = 8 Hz, 3α-H), 4.65 (1H, *m*, 23-H), 5.12 (1H, *m*, 24-H), 5.25 (1H, *m*, 7-H); MS *m/z* (rel. int.): 482 $[M]^+$ ($C_{32}H_{50}O_3$) (96), 467 $[M-Me]^+$ (100), 427 $[M-55]^+$ (2), 422 $[M-AcOH]^+$ (2), 407 $[M-Me-AcOH]^+$ (32), 297 [M – side chain-AcOH]$^+$ (3), 285(52), 255(*c*, 7), 187(11), 125(72), 83(10), 81(56), 70(5), 69(83), 68(3), 55(46).

Mercuric acetate oxidation of **1a**. Compound **1a** (15 mg) was dissolved in AcOH (2 ml), Hg $(OAc)_2$ (20 mg) added and the mixture stirred for 48 hr at room temp. and then filtered. The filtrate was freed of AcOH *in vacuo* and the residue purified by prep. TLC in $CHCl_3$–MeOH (99:1) to afford **2**, mp 167° (MeOH), 6 mg. UV λ_{max} nm: 230, 237, 246; IR ν_{max} cm^{-1}: 3020, 2950, 2920, 2860, 1705, 1450, 1375, 1365, 840; MS *m/z* (rel. int.): 436 $[M]^+$ ($C_{30}H_{44}O_2$) (3), 421 $[M-Me]^+$ (4), 381 $[M-55]^+$ (13), 311 [M – side chain]$^+$ (8), 269(10), 202(68), 125(14), 83(12), 81(11), 70(3), 69(35), 68(3), 55(30), 43(100), 41(32).

Hydrogenation of **1a**. Compound **1a** (50 mg) was dissolved in EtOH (100 ml) and hydrogenated (5% Pd–C, 20 mg) under pressure for 4 hr. After removal of the catalyst, the solvent was removed *in vacuo* and the residue purified by prep. TLC ($CHCl_3$–MeOH, 99:1) to afford two products with R_f 0.46 and 0.30 ($CHCl_3$). The fast moving compound gave **3**, mp 141° (MeOH), 32 mg, $[\alpha]_D$ – 30° ($CHCl_3$). IR ν_{max} cm^{-1}: 2955, 2930, 2860, 1700, 1640, 1465, 1385, 1365, 1260, 1170, 1060, 1040, 930, 840, 820; ^1H NMR: δ0.85 (6H, *d*, J = 6 Hz, 26-H_3, 27-H_3), 0.94 (3H, *s*, 18-H_3), 1.10 (3H, *s*, 19-H_3), 0.99 (6H, *s*, 28-H_3, 29-H_3), 1.04 (3H, *s*, 30-H_3), 1.90 (1H, *m*, 20-H), 2.10 (2H, *m*, 22-H_2), 2.80 (1H, *m*, 23-H), 2.30 (2H, *t*, J = 6 Hz, 2-H_2), 3.24 (1H, *t*, J = 9 Hz, 21-H), 3.95 (1H, *dd*, J = 11, 3 Hz, 21-H), 5.30 (1H, *m*, 7-H); MS *m/z* (rel. int.): 440 $[M]^+$ ($C_{30}H_{48}O_2$) (18), 425 $[M-Me]^+$ (100), 383 $[M-57]^+$ (37), 298 [M – Me – side chain]$^+$ (15), 271(4), 189(5), 187(5), 127(sc, 4), 85(44), 70(6), 69(31), 68(3), 57(45), 55(40), 43(48), 41(38).

The slow moving spot provided **4a**, mp 106° (MeOH), 15 mg, $[\alpha]_D$ – 9° ($CHCl_3$). IR ν_{max} cm^{-1}: 3450, 2950, 2860, 1705, 1640, 1465, 1382, 1365, 1050, 828; ^1H NMR: δ0.86 (6H, *d*, J = 6 Hz, 26-H_3, 27-H_3), 0.99 (3H, *s* 18-H_3), 1.10 (3H, *s*, 19-H_3), 1.02 (6H, *s*, 28-H_3, 29-H_3), 1.04 (3H, *s*, 30-H_3), 2.30 (2H, *t*, J = 6 Hz, 2-H_2), 3.65 (2H, *m*, $W_{1/2}$ = 16 Hz, 21-H_2), 5.20 (1H, *m*, 7-H); MS *m/z* (rel. int.): 442 $[M]^+$ ($C_{30}H_{50}O_2$) (4), 427 $[M-Me]^+$ (22), 409 $[M-Me-H_2O]^+$ (9), 313 [M – side chain]$^+$ (3), 298 [M – side chain – Me]$^+$ (3), 271(6), 189(2), 187(2), 129(side chain, 5), 85 (21), 71(38), 57(71), 55(61), 43(100).

Acetylation of **4a** (10 mg) with C_5H_5N–Ac_2O (0.5 ml each) overnight at room temp. gave **4b**, mp 134° (MeOH). IR ν_{max} cm^{-1}: 2950, 2865, 1735, 1705, 1465, 1382, 1365, 1250, 818; ^1H NMR: δ0.86 (6H, *d*, J = 6 Hz, 26-H_3, 27-H_3), 1.02 (3H, *s*, 18-H_3), 1.12 (3H, *s*, 19-H_3), 1.04 (6H, *s*, 28-H_3, 29-H_3), 1.06 (3H, *s*, 30-H_3), 2.06 (3H, *s*, -OAc), 2.31 (2H, *t*, J = 7 Hz, 2-H_2). 4.06 (2H, *m*, $W_{1/2}$ = 16 Hz, 21-H_2), 5.30 (1H, *m*, 7-H); MS *m/z* (rel. int.): 484$[M]^+$ ($C_{32}H_{52}O_3$) (1), 424 $[M-AcOH]^+$ (2), 313 [M – side chain]$^+$ (2), 271(4), 189(7), 171(8), 85(8), 71(8), 57(11), 55(32), 43(100).

3β-*Acetoxy*-21,23-*epoxytirulla*-7,24-*diene* (**1c**). Prep. TLC of *n*-hexane–C_6H_6 (1:1) fractions 145–168 afforded **1c**, 27 mg, R_f 0.34 (C_6H_6) which was found to be identical in all respects (mp, $[\alpha]_D$, IR, NMR, MS) with **1c** obtained from the alcohol **1b** of **1a**. (Found; C, 79.46; H, 10.25 %. $C_{32}H_{50}O_3$ requires: C, 79.66; H, 10.37 %.)

Hydrolysis of **1c**. Compound **1c** (10 mg) was refluxed with ethanolic KOH (5%, 10 ml, 5 hr) to give an alcohol which was similar to **1b** obtained from **1a** (mp, $[\alpha]_D$, co-TLC, IR, MS, NMR).

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