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TWO TRITERPENES FROM *DAVIDSONIA PRURIENS*

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Key Word Index—*Davidsonia pruriens*; Davidsoniaceae; bauerene derivatives; 3-oxo-bauer-7-en-28-oic acid; methyl 3-oxo-bauer-7-en-28-oate; 3β-hydroxy-bauer-7-en-28-oic acid.

Abstract—Two new triterpenoids, methyl 3-oxo-bauer-7-en-28-oate and 3β-hydroxy-bauer-7-en-28-oic acid, were isolated from the stem bark of *Davidsonia pruriens* and their structures were established by chemical and spectroscopic means.

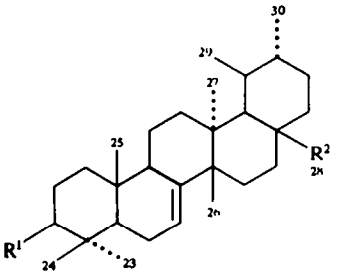
INTRODUCTION

Davidsonia pruriens F. Muell. is a monotypic genus in the family Davidsoniaceae. There is very little ethnomedical information available, the only apparent use being for jelly and preserves [1]. Several flavonoids were previously isolated from the leaves of this plant [2]. Our investigation of the chemical constituents of the stem bark of *D. pruriens* has resulted in the isolation and structure determination of three triterpenoids from the dichloromethane extract which showed weak activity in the KB [3] test system.

RESULTS AND DISCUSSION

Separation of the dichloromethane soluble fraction from the methanol extract of *D. pruriens* bark material by column chromatography provided compounds **1**, **2** and **3**. Methyl 3-oxo-bauer-7-en-28-oate (**1**) crystallized from chloroform–methanol as colourless crystals of molecular formula $C_{31}H_{48}O_3$. The IR spectrum of **1** showed the presence of two CO groups (1714 and 1700 cm^{-1}) and the ^1H NMR spectrum of **1** indicated the presence of two secondary methyl signals at δ0.847 and 1.013, five tertiary methyl signals at δ1.018, 1.030, 1.045, 1.100 and 1.115, a $-COCH_2-$ signal at δ2.751, a CO_2Me signal at δ3.688 and an olefinic H at δ5.453.

3-Oxo-bauer-7-en-28-oic acid (**2**) crystallized from petrol–chloroform as white crystals. The IR spectrum of **2** showed the presence of OH (3406 cm^{-1}) and CO groups (1710 and 1691 cm^{-1}), and the ^1H NMR spectrum of **2** showed two secondary methyl signals at δ0.86 and 1.007, five tertiary methyl signals at δ1.030, 1.040, 1.064, 1.102 and 1.110, $-COCH_2$ protons at δ2.761 and an olefinic H at δ5.463. Treatment of **2** with diazomethane gave a monomethyl ester, identical with methyl 3-oxo-bauer-7-en-28-oate (**1**), thereby indicating the presence of a carboxyl group in **2**. The mass spectrum of **2** exhibited the retro-Diels–Alder fragmentation in the C ring of a bauer-7-en-3-one (**4**) derivative, giving rise to a base peak at *m/z* 245 [4]. The formation of fragments *m/z* 218 and 245 from **2** demonstrated the absence of a carboxyl group in rings A and B, as well as the presence of a keto group at C-3. Comparison of the physical and spectral data with those of known 3-oxo-bauer-7-en-x-oic acids indicated that the



	R^1	R^2
1	O	COOMe
2	O	COOH
3	β-OH	COOH
4	O	Me
5	α-OH	COOH
6	α-OH	CH_2OH
7	β-OH	CH_2OH
8	β-OAc	CH_2OAc
9	H_2	Me
10	β-OH	Me
11	O	CHO
12	β-OH	COOMe
13	β-OH	CD_2OH
14	β-OAc	CD_2OAc
15	H_2	CD_2H
16	β-OH	CD_2H
17	β-OAc	COOH

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mp and the mass spectral data of compound **2** resembled very closely those of terebenthifolic acid. However, the ^{1}H NMR spectrum showed some differences [5]. No authentic sample of this compound was available for direct comparison. In addition, the original structure assignment of terebenthifolic acid had been based primarily on mass spectral data with little other detailed evidence.

Sodium borohydride reduction of isolate **2** afforded the 3β-hydroxy (**3**) and 3α-hydroxy (**5**) derivatives, but the latter did not correspond with myrtifolic acid which was proposed to be 3α-hydroxy-bauer-7-en-28-oic acid (**5**) [6]. It therefore became necessary to establish the structure of **2** unambiguously through chemical and spectroscopic methods.

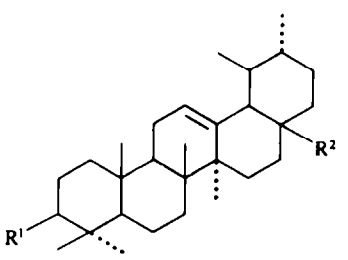
Methylation of **2** followed by reduction with lithium aluminium hydride resulted in the 3α,28-diol **6** and the 3β,28-diol **7**, as supported by the loss of the two CO absorptions and the presence of hydroxyl groups at 3376 cm^{-1} in the IR spectra, and a shift of the base peak to *m/z* 247 in the mass spectra. In addition, the presence of an AB-type signal for the hydroxymethylene, and the multiplet of the –CHOH at δ3.344 and 3.464 for compound **6**, and at δ3.342 and 3.249 for compound **7**, respectively, were noted in the ^{1}H NMR spectra. Acetylation of compound **7** with acetic anhydride in pyridine afforded a diacetate **8** which was reduced with Li in ethylenediamine [7] to afford bauer-7-ene (**9**), bauer-7-en-3β-ol (**10**) and the 3β-diol (**7**). Compound **10** was identical with an authentic sample of β-bauerenol by means of mixed mp, TLC, IR, mass and ^{1}H NMR spectral data. This confirmed the presence of a bauerene skeleton and a carbonyl group at C-3 in **2**.

It remained therefore to locate the carboxyl group on ring C, D or E. The oxidation of **7** with CrO_3 in pyridine afforded **11** which in its ^{1}H NMR spectrum exhibited a singlet for a formyl proton at δ9.457, and in its IR spectrum showned diagnostic aldehyde bands (2870 and 2750 cm^{-1}). The possibility of a carboxyl group at either C-19 or C-20 in **2** was therefore eliminated since a doublet for the aldehyde proton would be anticipated in these cases. Methylation of **3** with diazomethane afforded a monomethyl ester **12** whose ^{1}H NMR spectrum showed the presence of a CO_2Me group at δ3.682 and whose IR spectrum showed the shift of the carbonyl absorption band to 1724 cm^{-1}. Reduction of **12** with $LiAlD_4$ yielded a deuterated diol **13** whose ^{1}H NMR spectrum indicated the disappearance of the AB-type signal of the hydroxymethylene group by comparison with those of compound **7**. Moreover, the deuterated diol **13**, on acetylation with acetic anhydride in pyridine, gave rise to a deuterated diacetate **14**, which was reduced with Li in ethylenediamine to the deuterated bauerene analogs **15**, **16** and **13**. The ^{1}H NMR spectrum of **16** showed a decrease in the intensity of the methyl singlet at δ1.037.

An indication of a plausible assignment of the methyl resonances of β-bauerenol was achieved on the basis of pseudo-contact shifts introduced by Eu(dpm)$_3$ [tris(2,2,6,6- tetramethyl- 3,5 -heptanedionato)europium] and from two-dimensional NOE techniques. On increasing the amount of Eu(dpm)$_3$, the spectrum was resolved so that at [L]/[S] = 0.5, the signals of all eight methyls were observed distinctly. Assignment was based on the observation of the Drieding model of the conformation of bauerenyl acetate as assigned by X-ray crystallography [8], i.e. chair–boat–skew–chair–boat for rings A, B, C, D and E, respectively; ring A/B *trans* junction with H-5α and C-25β; H-9α; ring C/D *trans* associated with C-26β and C-27α; ring D/E *cis* coupled with H-18β and C-28β and equatorial 30α-Me, as well as on the McConnel–Robertson equation [9]. The magnitude of the induced shift of the methyl groups in $CDCl_3$ was in the order: 24 > 23 ⋙ 25 > 26 > 28 > 29 ≥ 27 ≃ 30, which was different from a previous report [10]. This permitted the tentative assignment of the signals at δ0.965, 0.855, 0.743, 0.993, 1.056, 1.037, 0.941 and 0.903 to the methyl groups C-23, 24, 25, 26, 27, 28, 29 and 30, respectively.

Furthermore, a two-dimensional NOE experiment [11], in combination with a lanthanide-induced shift reagent was applied for substantiation of the above assignments. In the absence of paramagnetic influences, NOEs were observed between the proton adjacent to the hydroxyl group (δ3.253) and the methyl signal at δ0.965, which further displayed two NOEs with the allylic protons (δ2.164) and the methyl signal at δ0.855, and vice versa. The resonance at δ0.855 additionally gave a weak NOE with the H-2 axial proton (δ1.971) and a strong NOE with the methyl resonance at δ0.743. This latter signal also showed an NOE with the methyl signal at δ0.993. Therefore, the resonances at δ0.965, 0.855, 0.743 and 0.993 were assigned to the 23-, 24-, 25- and 26-methyls, respectively. The methyl–methyl NOE was also observed between δ0.941 and 0.903. According to the increased induced shift of the methyl resonance at δ0.941 than that at 0.903 in the presence of the saturated concentration of Eu(dpm)$_3$, the resonances at δ0.941 and 0.903 were assigned to the 29- and 30-methyls, respectively. It is noteworthy that the 26-methyl resonance and the signal at δ1.037 coincidently exhibited the same NOE with the signal at δ1.296. Thus, the signal at δ1.037 should be assigned to the 28-methyl. One methyl resonance, at δ1.056, showed no significant NOE, and was assigned to the 27-methyl.

Inspection of the methyl resonances of bauerenol and comparison with the deuterated analog **16** which exhibited a decrease in the intensity of the signal at δ1.037 assigned to the 28-methyl protons, led to the tentative location of the carboxyl group of compound **2** at C-28. This was confirmed by isomerization of the acetylation product **17**, which with dry hydrogen chloride in phenol [12] afforded **18** and **19**. The ^{1}H NMR spectrum of **18** showed the presence of phenyl, olefinic, –CHOAc and acetyl protons at δ7.176, 5.318, 4.506 and 2.051, respectively. The IR spectrum exhibited CO groups at 1741 and



	R¹	R²
18	β-OAc	$COOC_6H_5$
19	Δ^2	$COOC_6H_5$
20	β-OH	COOH

1732 cm^{-1} and the mass spectrum revealed a molecular ion at *m/z* 574. Compound **19** showed only the presence of phenyl and three olefinic protons at δ7.191 and 5.346, respectively, in the ^1H NMR spectrum, the presence of one CO absorption at 1750 cm^{-1} in the IR spectrum and an intense molecular ion at *m/z* 514. In the presence of a mineral acid under anhydrous conditions isomerization and phenolic ester formation were observed as **18** underwent elimination of acetic acid to yield **19**. It was concluded therefore that **18** and **19** were phenyl-3β-acetoxy-urs-12-en-28-oate and phenyl-ursa-2,12-dien-28-oate, respectively. The structure of compound **18** was further confirmed by saponification with potassium hydroxide in ethylene glycol to afford **20** which was identical with an authentic sample of ursolic acid by means of mixed mp, TLC, IR, MS and ^1H NMR.

The previously described experiments succinctly demonstrate that **2** possessed a 28-carboxylic group, leading to a structure assignment as 3-oxo-bauer-7-en-28-oic acid or D:C 3-oxo-friedours-7-en-28-oic acid. Overall, the chemical and spectral evidence unambiguously established the structures of the other two triterpenes isolated from *D. pruriens*, namely methyl 3-oxo-bauer-7-en-28-oate (**1**) and 3β-hydroxy-bauer-7-ene-28-oic acid (**3**), which were identical with the methylation and sodium borohydride reduction products, respectively of 3-oxo-bauer-7-en-28-oic acid (**2**).

Terebenthifolic acid isolated from *Schinus terebenthifolius* probably has the same structure as compound **2**. Examination of the original ^1H NMR spectrum indicates that the differences in the ^1H NMR spectral data may be due to the unrealized presence of an impurity. Comparison of 3α-hydroxy-bauer-7-en-28-oic acid with an authentic sample of myrtifolic acid [6], indicated that they were different compounds. Analysis of the spectral data, and a recent report on the stereochemistry of bauerene-type triterpenes [8] suggested that the axial carboxylic group at C-13 was not as sterically hindered as expected by Gunasekera *et al.* [6], since rings D and E were *cis*-fused in the bauerene skeleton, and ring E was in a boat conformation with an equatorial 30α-Me. Consideration should therefore be given to a revision of the structure of myrtifolic acid, with the possibility that the carboxyl group is located at C-13.

EXPERIMENTAL

Plant material. The stem bark material of *Davidsonia pruriens* F. Muell was collected in Australia in May 1980. A herbarium specimen is deposited at the Herbarium of the National Arboretum, Washington, DC.

Extraction and isolation. Air-dried milled stem bark material (90 kg) was defatted with petrol followed by successive partitioning with petrol and CH_2Cl_2 from the aq. MeOH extract with an increasing amount of H_2O. Removal of the CH_2Cl_2 afforded a brown viscous solid (68.69 g) which was chromatographed over silica gel G60. Gradient elution with petrol–$CHCl_3$, $CHCl_3$ and $CHCl_3$–MeOH gave 84 fractions (250 ml each). Fractions 9–12, on crystallization from $CHCl_3$–MeOH, yielded 98 mg of colourless crystals of **1**. Fractions 15–24, on crystallization from petrol–MeOH, afforded 2.58 g of white crystals **2**. Fractions 25–31 (4.25 g) were rechromatographed on silica gel G60 with petrol–$CHCl_3$, $CHCl_3$ and $CHCl_3$–MeOH to give 15 fractions (150 ml each). Fractions 8–11, on crystallization with $CHCl_3$, yielded 51.9 mg of **3**.

Methyl 3-oxo-bauer-7-en-28-oate (**1**). Colourless crystals, mp 236°; $[\alpha]_D^{25}$ – 22.4°(*c* 0.31; $CHCl_3$); R_f $CHCl_3$: 0.5; UV λ_{max}^{EtOH} (log ε): 209 (3.54); IR ν_{max}^{KBr} cm^{-1}: 2967, 2931 (aliphatic CH) and 1714, 1700 (C=O); ^1H NMR (360 MHz, $CDCl_3$, TMS as internal standard): δ0.847 (3H, *d*, *J* = 5.7 Hz), 1.013 (3H, *br s*), 1.018 (3H, *s*), 1.030 (3H, *s*), 1.045 (3H, *s*), 1.100 (3H, *s*), 1.115 (3H, *s*), 2.751 (1H, *ddd*, *J* = 19.8, 19.8, 5.4 Hz, O=C–CH_2–), 3.688 (3H, *s*, CO_2Me) and 5.453 (1H, *m*, –C=CH); EIMS 70 eV, *m/z* (rel. int.): 468 $[M]^+$ (9), 453 (11), 393 (10), 272 (3), 271 (5), 257 (13), 249 (36), 245 (67) and 218 (15). Found *m/z* 468.3601 ($C_{31}H_{48}O_3$ requires 468.3604).

3-Oxo-bauer-7-en-28-oic acid (**2**). White needles, mp 274°; $[\alpha]_D^{25}$ – 22.4° (*c* 0.25; $CHCl_3$); R_f $CHCl_3$–MeOH (49:1): 0.48; UV λ_{max}^{EtOH} nm (log ε): 209 (3.7); IR ν_{max}^{KBr} cm^{-1}: 3406 (OH), 2965, 2951 (aliphatic CH), 1710 (C=O) and 1691 (CO_2H); ^1H NMR (360 MHz, $CDCl_3$, TMS as internal standard): δ0.86 (3H, *d*, *J* = 4.29 Hz), 1.007 (3H, *br s*), 1.030 (3H, *s*), 1.040 (3H, *s*), 1.064 (3H, *s*), 1.102 (3H, *s*), 1.110 (3H, *s*), 2.761 (1H, *ddd*, *J* = 14.8, 14.8, 5.4 Hz, O=C–CH_2–), 5.463 (1H, *m*, –C=CH); EIMS 70 eV, *m/z* (rel. int.): 454 $[M]^+$ (22), 439 (27), 272 (5), 271 (5), 257 (20), 245 (100), 235 (62) and 218 (14).

3β-Hydroxy-bauer-7-en-28-oic acid (**3**). White needles, mp 305–308°; $[\alpha]_D^{25}$ – 8.8° (*c* 0.25; $CHCl_3$); R_f $CHCl_3$–MeOH (49:1): 0.33; UV λ_{max}^{MeOH} nm (log ε): 208 (3.84), 240 (2.78); IR ν_{max}^{KBr} cm^{-1}: 3466 (OH), 1700 (C=O); ^1H NMR (360 MHz, $CDCl_3$, TMS as internal standard): δ0.763 (3H, *s*), 0.871 (6H, *br s*), 0.982 (3H, *s*), 1.046 (3H, *s*), 1.081 (6H, *br s*), 3.246 (1H, *dd*, *J* = 9.3, 3.9 Hz, HO–CH–) and 5.424 (1H, *m*, –C=CH); EIMS 70 eV, *m/z* (rel. int.): 456 $[M]^+$ (32), 441 (51), 423 (28), 274 (5), 273 (4), 259 (11), 247 (61), 235 (45) and 220 (8).

Reduction of 3-oxo-bauer-7-en-28-oic acid (**2**) *with* $NaBH_4$. 3-Oxo-bauer-7-en-28-oic acid (**2**, 100 mg) was reduced with $NaBH_4$ (100 mg) in MeOH (5 ml) for 1 hr at room temp., followed by the usual work-up, to afford a mixture (102.2 mg) which was separated on a silica gel column (12 g) eluting with petrol–$CHCl_3$ (1:1). Ninety-seven 10 ml fractions were collected. Fractions 25–52 gave 3α-hydroxy-bauer-7-en-28-oic acid (**4**, 9.3 mg) and fractions 63–97 gave **3** (68.7 mg) which was identical with the isolated 3β-hydroxy-bauer-7-en-28-oic acid by means of mmp, TLC, IR, NMR and MS. 3α-Hydroxy-bauer-7-en-28-oic acid (**4**) was a white solid, mp 320–321°; R_f $CHCl_3$–MeOH (49:1): 0.4; IR ν_{max}^{KBr} cm^{-1}: 2948 (aliphatic CH), 1712 (CO_2H); ^1H NMR (360 MHz, $CDCl_3$, TMS at internal standard): δ0.777 (3H, *s*) 0.869 (3H, *d*, *J* = 5.1 Hz), 0.915 (3H, *s*), 0.937 (3H, *s*), 1.042 (6H, *s*), 1.075 (3H, *s*), 3.471 (1H, *t*, *J* = 2.6 Hz, HO–CH–), and 5.415 (1H, *m*, –C=CH); EIMS 70 eV, *m/z* (rel. int.): 456 $[M]^+$ (6), 441 (9), 423 (10), 259 (5), 247 (22) and 235 (16).

Methylation of 3-oxo-bauer-7-en-28-oic acid (**2**) *with* CH_2N_2. 3-Oxo-bauer-7-en-28-oic acid (**2**, 73.6 mg) was treated with excess ethereal CH_2N_2 soln to afford compound **1** (84.6 mg) identical with the isolated methyl 3-oxo-bauer-7-en-28-oate.

Reduction of methyl 3-oxo-bauer-7-en-28-oate (**1**) *with* $LiAlH_4$. $LiAlH_4$ (73 mg) was added slowly to a soln of methyl 3-oxo-bauer-7-en-28-oate (**1**, 73.6 mg) in dry THF (3 ml) and the mixture heated under reflux at 65–66° for 4 hr and allowed to stand overnight at room temp. Work-up in the usual way gave a mixture (65.5 mg) which was separated on a silica gel column (20 g) eluting with petrol–$CHCl_3$ (9:1). Two hundred 20 ml fractions were collected. Fractions 39–62 gave 3α,28-dihydroxy-bauer-7-ene (**6**, 15.2 mg); mp 222°; R_f hexane–EtOAc (3:1): 0.34; IR ν_{max}^{KBr} cm^{-1}: 3473 (OH), 2954, 2944, 2927, 2870 (aliphatic CH); ^1H NMR (360 MHz, $CDCl_3$, TMS as internal standard): δ0.769 (3H, *s*), 0.87 (3H, *d*, *J* = 7.4 Hz), 0.909 (3H, *d*, *J* = 4.1 Hz), 0.937 (3H, *s*), 0.998 (3H, *s*), 1.020 (3H, *s*), 1.030 (3H, *s*), 3.344 (2H, AB, J_{AB} = 10.4 Hz, $HOCH_2$–), 3.464 (1H, *d*, *J* = 4.3 Hz, HO–CH–) and 5.434 (1H, *d*, *J* = 0.4 Hz, –C=CH); EIMS 70 eV.

m/z (rel. int.): 442 $[M]^+$ (4), 427 (3), 411 (1), 393 (5), 273 (2), 259 (3), 247 (18), 229 (14), 221 (4) and 220 (2). Fractions 126–200 afforded 3β,28-dihydroxy-bauer-7-ene (**7**, 50.06 mg); mp 225–226°; R_f hexane–EtOAc (3:1): 0.25; IR ν_{max}^{KBr} cm^{-1}: 3376 (OH), 2949, 2929, 2867 (aliphatic CH); ^1H NMR (360 MHz, $CDCl_3$, TMS as internal standard): δ0.745 (3H, *s*), 0.858 (3H, *s*), 0.909 (3H, *d*, *J* = 5 Hz), 0.970 (3H, *br s*), 0.992 (3H, *s*), 1.016 (3H, *s*), 1.031 (3H, *s*), 3.249 (1H, *dd*, *J* = 11.2, 3.7 Hz, HO–CH–), 3.342 (2H, AB, J_{AB} = 10.8 Hz, $HOCH_2$–) and 5.443 (1H, *m*, –C=CH); EIMS 70 eV, *m/z* (rel. int.): 442 $[M]^+$ (13), 427 (8), 411 (8), 393 (3), 273 (4), 259 (10), 247 (100), 229 (48), 221 (12) and 220 (3).

Acetylation of 3β,28-Dihydroxy-bauer-7-ene (**7**). Compound **7** (79.3 mg) was dissolved in C_5H_5N–Ac_2O (2 ml, 1:1) and left for 12 hr at room temp. Work-up gave 3β,28-diacetoxy-bauer-7-ene (**8**, 82.1 mg); mp > 350°; R_f hexane–EtOAc (10:1): 0.31; IR ν_{max}^{KBr} cm^{-1}: 2951 (aliphatic CH), 1738 (ester C=O); ^1H NMR (360 MHz, $CDCl_3$, TMS as internal standard): δ0.769 (3H, *s*), 0.852 (3H, *s*) 0.911 (3H, *d*, *J* = 5.8 Hz), 0.933 (3H, *s*), 0.976 (3H, *d*, *J* = 6.95 Hz), 0.991 (3H, *s*), 1.022 (3H, *s*), 2.057 (3H, *s*, $\underline{Me}CO_2CH$–), 2.075 (3H, *s*, $\underline{Me}CO_2CH_2$–), 3.825 (2H, AB, J_{AB} = 10.8 Hz, $AcOC\underline{H}_2$–), 4.519 (1H, *dd*, *J* = 10.8, 4.3 Hz, $AcOC\underline{H}$–), 5.431 (1H, *m*, –C=CH); EIMS 70 eV, *m/z* (rel. int.): 526 (M^+, 2), 511 (2), 451 (8), 316 (1), 301 (3), 289 (35) and 263 (2).

Metal–amine reduction of 3β,28-diacetoxy-bauer-7-ene (**8**). Lithium wire (80 mg) was added slowly to a soln of 3β,28-diacetoxy-bauer-7-ene (**8**, 82.1 mg) in ethylenediamine (8 ml) and the mixture heated under reflux for 1 hr. The excess lithium wire was destroyed with MeOH. After standard work-up, the crude product (74.0 mg) was separated on a silica gel column (25 g) eluting with a petrol–$CHCl_3$ gradient. Eighty fractions (50 ml each) were collected. Fractions 1–2 gave bauerene (**9**, 7.2 mg); mp 225–228° (lit. 108–109° [6], 247° [13]; R_f hexane–EtOAc (3:1): 0.78; IR ν_{max}^{KBr} cm^{-1}: 3047 (olefinic CH), 2984, 2949, 2865 (aliphatic CH); ^1H NMR (360 MHz, $CDCl_3$, TMS as internal standard): δ0.741 (3H, *s*), 0.841 (3H, *s*), 0.879 (3H, *s*), 0.902 (3H, *d*, *J* = 5.8 Hz), 0.954 (3H, *br s*), 0.994 (3H, *s*), 1.035 (3H, *s*), 1.055 (3H, *s*) and 5.404 (1H, *m*, –C=CH); EIMS 70 eV, *m/z* (rel. int.): 410 $[M]^+$ (22), 395 (23), 257 (5), 243 (21), 231 (100), 205 (10) and 204 (5). Fractions 49–51 gave white needles of β-bauerenol (**10**, 4.6 mg); mp 208° (lit. 207–208° [14]); R_f hexane–EtOAc (3:1): 0.4; IR ν_{max}^{KBr} cm^{-1}: 3378 (OH), 2974, 2867 (aliphatic CH); ^1H NMR (360 MHz, $CDCl_3$, TMS as internal standard): δ0.743 (3H, *s*, 25-Me), 0.855 (3H, *s*, 24-Me), 0.903 (3H, *d*, *J* = 5.9 Hz, 30-Me), 0.941 (3H, *br s*, 29-Me), 0.965 (3H, *s*, 23-Me), 0.993 (3H, *s*, 26-Me), 1.037 (3H, *s*, 28-Me), 1.056 (3H, *s*, 27-Me), 3.253 (2H, *dd*, *J* = 11.0, 3.9 Hz, HOCH–) and 5.422 (1H, *m*, –C=CH); EIMS 70 eV, *m/z* (rel. int.): 426 $[M]^+$ (3), 411 (21), 393 (6), 273 (3), 259 (18), 247 (100), 229 (47) and 205 (18), which was identical with an authentic sample of β-bauerenol (mmp, ^1H NMR, IR, MS and TLC comparison). Fractions 53–80 afforded 3β,28-dihydroxy-bauer-7-ene (**7**, 18.6 mg).

CrO_3 oxidation of 3β,28-dihydroxy-bauer-7-ene (**7**). Compound **7** (35.3 mg) in C_5H_5N (3 ml) was oxidized with CrO_3 (30 mg). Usual work up and crystallization from EtOAc yielded bauer-7-ene-3,28-dione (**11**, 18.2 mg) as white needles; mp 215°; R_f hexane–EtOAc (3:1): 0.46; IR ν_{max}^{KBr} cm^{-1}: 2949 (aliphatic CH), 2870, 2750 (aldehydic CH), 1710 (C=O); ^1H NMR (360 MHz, $CDCl_3$, TMS as internal standard): δ0.840 (3H, *s*), 0.998 (3H, *d*, *J* = 6.7 Hz), 1.018 (3H, *s*), 1.050 (3H, *s*), 1.059 (3H, *s*), 1.075 (3H, *s*), 1.120 (3H, *s*), 2.763 (1H, *ddd*, *J* = 14.6, 14.6 and 5.6 Hz, O=C–CH_2–), 5.480 (1H, *d*, *J* = 3.04 Hz, –C=CH) and 9.457 (1H, *s*, O=CH–); EIMS 70 eV, *m/z* (rel. int.): 440 (3), 439 (7), 438 $[M]^+$ (22), 423 (14), 409 (19), 395 (19), 272 (10), 271 (23), 257 (30), 245 (84), 219 (32) and 218 (6).

Methylation of 3β-hydroxy-bauer-7-en-28-oic acid (**3**) *with* CH_2N_2. Compound **3** (102.1 mg) was treated with excess ethereal methanolic CH_2N_2 at room temp. overnight. Evaporation afforded a white solid of methyl 3β-hydroxy-bauer-7-en-28-oate (**12**, 96.9 mg); mp 182°; R_f hexane–EtOAc (3:1): 0.35; IR ν_{max}^{KBr} cm^{-1}: 3484 (OH), 2959, 2949, 2870 (aliphatic CH) and 1724 (ester C=O); ^1H NMR (360 MHz, $CDCl_3$, TMS as internal standard): δ0.750 (3H, *s*), 0.840 (3H, *d*, *J* = 5.7 Hz), 0.857 (3H, *s*), 0.968 (3H, *s*), 1.004 (3H, *d*, *J* = 6.5 Hz), 1.027 (3H, *s*), 1.060 (3H, *s*), 3.248 (1H, *dd*, *J* = 11.2, 3.9 Hz, HO–CH–), 3.682 (3H, *s*, $-CO_2Me$) and 5.405 (1H, *m*, –C=CH); EIMS 70 eV, *m/z* (rel. int.): 470 $[M]^+$ (52), 455 (55), 437 (11), 274 (4), 273 (4), 259 (13), 249 (66), 247 (61) and 220 (20).

Reduction of **12** *with* $LiAlD_4$. $LiAlD_4$ (97.8 mg) was added slowly to a soln of **11** (90.1 mg) in dry THF (3 ml) and the mixture heated under reflux for 4 hr. Work-up in the usual way afforded 3β,28-hydroxy-bauer-7-ene-28-d_2 (**13**, 75.2 mg); mp 206°; R_f hexane–EtOAc (3:1): 0.25; IR ν_{max}^{KBr} cm^{-1}: 3369 (OH) and 2952, 2867 (aliphatic CH); ^1H NNR (360 MHz, $CDCl_3$, TMS as internal standard): δ0.744 (3H, *s*), 0.858 (3H, *s*), 0.902 (3H, *d*, *J* = 5.5 Hz), 0.909 (3H, *d*, *J* = 5.6 Hz), 0.969 (3H, *s*), 0.992 (3H, *s*), 1.015 (3H, *s*), 1.029 (3H, *s*), 3.249 (1H, *dd*, *J* = 11.3, 3.9 Hz, HO–CH–) and 5.442 (1H, *d*, *J* = 3.0 Hz, –C=CH); EIMS 70 eV, *m/z* (rel. int.): 444 $[M]^+$ (24), 429 (11), 411 (9), 393 (2), 273 (1), 271 (2), 259 (7) 247 (68), 229 (31), 221 (4) and 220 (2).

Acetylation of **13**. Compound **13** (72.1 mg) was treated with Ac_2O–C_5H_5N (1:1, 3 ml) at room temp. for 48 hr. Work-up gave 3β,28-diacetoxy-bauer-7-ene-28-d_2 (**14**, 72.7 mg); mp 192–195°; R_f petrol–$CHCl_3$ (4:1); 0.36; EIMS 70 eV, *m/z* (rel. int.): 528 $[M]^+$ (46), 513 (16), 453 (47), 316 (5), 301 (12), 289 (84), 263 (2) and 262 (3).

Metal–amine reduction of **14**. Lithium wire (72.1 mg) was added slowly to a soln of compound **14** (71.6 mg) in ethylenediamine (5 ml) and the mixture refluxed under anhydrous conditions for 1 hr. The excess lithium wire was destroyed with MeOH. Work-up gave a crude product (70.5 mg) which was separated on a silica gel column (10 g) eluting with a petrol–$CHCl_3$ gradient. Two hundred and sixty-five fractions (100 ml) were collected. Fraction 1 gave bauer-7-ene-28-d_2 (**15**, 15.6 mg); mp 220–221°; R_f CH_2Cl_2: 0.86; ^1H NMR (360 MHz, $CDCl_3$, TMS as internal standard): δ0.741 (3H, *s*), 0.841 (3H, *s*), 0.879 (3H, *s*), 0.902 (3H, *d*, *J* = 5.8 Hz), 0.954 (3H, *s*), 0.993 (3H, *s*), 1.035 (1H, *s*), 1.054 (3H, *s*) and 5.404 (1H, *m*, –C=CH); EIMS 70 eV, *m/z* (rel. int.): 412 $[M]^+$ (4), 397 (4), 257 (2), 243 (7), 231 (38), 207 (4), 205 (3) and 204 (2). Fractions 101–114 afforded white solid of 3β-hydroxy-bauer-7-ene-28-d_2 (**16**, 6.9 mg); mp 198–200°; R_f CH_2Cl_2: 0.33; ^1H NMR (360 MHz, $CDCl_3$, TMS as internal standard): δ0.743 (3H, *s*), 0.858 (3H, *s*), 0.915 (3H, *d*, *J* = 3.5 Hz), 0.941 (3H, *s*), 0.966 (3H, *s*), 0.992 (3H, *s*), 1.035 (1H, *s*), 1.054 (3H, *s*), 3.247 (1H, *dd*, *J* = 11.2, 4 Hz, HO–CH–) and 5.418 (1H, *m*, –C=CH); EIMS 70 eV, *m/z* (rel. int.): 428 $[M]^+$ (18), 413 (12), 395 (3), 273 (3), 259 (11), 247 (51), 229 (29), 220 (3) and 207 (10). Fractions 258–265 gave white needles of compound **13** (18.7 mg).

Acetylation of 3β-hydroxy-bauer-7-en-28-oic acid (**3**). Compound **3** (7.4 mg) was treated with Ac_2O–C_5H_5N (1:1, 1 ml) at room temp. for 24 hr. Work-up in the usual way afforded 3β-acetoxy-bauer-7-en-28-oic acid (**17**, 6.96 mg) as a white solid; mp 276–280°; R_f $CHCl_3$: 0.58; IR ν_{max}^{KBr} cm^{-1}: 2947, 2871 (aliphatic CH), 1733 (ester C=O) and 1691 (CO_2H); ^1H NMR (360 MHz, $CDCl_3$, TMS as internal standard): δ0.763 (3H, *s*), 0.864 (6H, *br s*), 0.932 (3H, *s*), 1.037 (3H, *s*), 1.070 (6H, *br s*), 2.058 (3H, *s*, $MeCO_2$–), 4.510 (1H, *dd*, *J* = 10.7, 4.5 Hz, AcO–CH–) and 5.400 (1H, *m*, –C=CH); EIMS 70 eV, *m/z* (rel. int.): 498 $[M]^+$ (3), 483 (3), 423 (5), 316 (1), 301 (1), 289 (4), 262 (1), and 235 (6).

Isomerization of 3β-acetoxy-bauer-7-en-28-oic acid (**17**). Compound **17** (90.5 mg) and phenol (1.8 g) were satd with dry HCl at 110° for 2 hr. The soln was cooled, poured into aq. 5%

KOH (50 ml) and extracted with CH_2Cl_2. Preparative TLC of the product in CH_2Cl_2 followed by crystallization from CH_2Cl_2–MeOH gave phenyl 3β-acetoxy-urs-12-en-28-oate (**18**, 1.96 mg) and phenyl ursa-2,12-dien-28-oate (**19**, 6.33 mg.). Compound **18** was a white powder; mp 70°; R_f petrol–CH_2Cl_2 (4:3): 0.19; IR ν_{max}^{KBr} cm^{-1}: 2924, 2874 (aliphatic CH), 1741, 1732 (ester C=O), 1246 (C–C(=O)–O), 1195 (O–C–) and 1182 (O–C=C); ^{1}H NMR (360 MHz, $CDCl_3$, TMS as internal standard): δ0.858 (3H, *s*), 0.875 (3H, *s*), 0.884 (3H, *s*), 0.904 (3H, *s*), 0.963 (3H, *d*, *J* = 3.08 Hz), 0.990 (3H, *d*, *J* = 2.9 Hz), 1.129 (3H, *s*), 2.051 (3H, *s*, $MeCO_2$–), 4.506 (1H, *dd*, *J* = 9.9, 6.2 Hz, AcO–CH–), 5.318 (1H, *m*, –C=CH) and 7.176 (5H, *m*, arom. H); EIMS 70 eV, *m/z* (rel. int.): 574 $[M]^+$ (2), 514 (2), 487 (1), 453 (38), 393 (6), 324 (32), 250 (1), 249 (5) and 203 (47). Compound **19** was obtained as white needles; mp 209–211°; R_f petrol–CH_2Cl_2 (4:3): 0.53; IR ν_{max}^{KBr} cm^{-1}: 2927, 2871 (aliphatic CH), 1750 (ester C=O), 1596 (C=C), 1199 (O–C–), 1181 (O–C–C); ^{1}H NMR (360 MHz, $CDCl_3$, TMS as internal standard): δ0.881 (3H, *s*), 0.899 (3H, *d*, *J* = 2.5 Hz), 0.917 (3H, *d*, *J* = 2.89 Hz), 0.960 (3H, *s*), 0.977 (3H, *s*), 1.030 (3H, *s*), 1.083 (3H, *s*), 5.346 (3H, *m*, –C=CH) and 7.191 (5H, *m*, arom. H); EIMS 70 eV, *m/z* (rel. int.): 514 $[M]^+$ (58), 499 (7), 421 (2), 393 (97), 377 (9), 309 (6), 255 (7), 241 (13), 239 (9), 229 (9), 215 (11), 213 (16), 207 (12), 205 (16), 203 (22), 201 (15), 189 (18), 187 (30) and 173 (49).

Saponification of phenyl 3β-acetoxy-urs-12-en-28-oate (**18**) *to ursolic acid* (**20**). Compound **18** (1.96 mg) was refluxed for 15 hr with KOH (500 mg) in ethylene glycol (5 ml). Work up and purification by preparative TLC yielded 1.33 mg of **20** which was identical with ursolic acid by means of mmp, TLC, IR, MS and ^{1}H NMR.

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