Pergamon

0031–9422(95)00105–O

Phytochemistry, Vol. 39, No. 6, pp. 1399–1402, 1995

0031–9422/95 $9.50 + 0.00

DITERPENOID LACTONES FROM THE ROOTS OF *GYNOCARDIA ODORATA*

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(*Received in revised form* 4 *January* 1995)

Key Word Index—*Gynocardia odorata*; Flacourtiaceae; roots; entkauranoids; diterpenoid lactones; 16α-hydroxy-ent-kauran-19 → 20-olide; ent-kaur-16(17)-en-19 → 20-olide; ent-kaur-15(16)-en-19 → 20-olide.

Abstract—Three new diterpenoid lactones, odolide iso-odolide and hydroxy odolide, isolated from the roots of *Gynocardia odorata* have been characterized as ent-kaur-15(16)-en-19 → 20-olide, ent-kaur-16(17)-en-19 → 20-olide and 16α-hydroxy-ent-kauran-19 → 20-olide on the basis of spectral and chemical analysis. The higher terpenoids isolated were odolactone, trichadenic acid-A and β-sitosterol.

INTRODUCTION

The isolation of three triterpenoid lactones [1] from the bark of *Gynocardia odorata* [2] have been reported. The structure of odolactone was subsequently revised on the basis of carbon–carbon connectivity 2D NMR studies and this led to the revision of the structures of the series of compounds discussed in a later communication [3]. We now report on the isolation of three new diterpenoid lactones each with an ent-kaurane skeleton along with odolactone, trichadenic acid-A and β-sitosterol.

RESULTS AND DISCUSSION

The neutral part of the benzene extract of the roots of *G. odorata* was chromatographed over silica gel. The less polar fraction was identified as odolactone (**1**) [1, 3]. Further elution with solvents of increasing polarity afforded first a mixture of odolide (**2a**) and iso-odolide (**2b**) followed by hydroxy odolide (**3**) and β-sitosterol. The acidic fraction of the benzene extract yielded trichadenic acid-A(**4**) [1, 3].

The mixture of **2a** and **b** could not be separated by column chromatography and GLC showed the ratio of these two to be components 4:1. The mixture was crystallized from acetone to furnish fine flake-like crystals, $C_{20}H_{28}O_2$, $[M]^+$ *m/z* 300 mp 131–132°, $[\alpha]_D^{CHCl_3}$ − 72°. The mixture showed a single spot on TLC and gave a yellow colouration with TNM indicating the presence of double bonds. The IR spectrum showed the presence of a δ-lactone ring (1725 and 1150 cm^{-1}) and an exocyclic (3010, 1640 and 880 cm^{-1}) along with a trisubstituted (810 cm^{-1}) double bond. The ^1H NMR spectrum showed a sharp peak at δ0.90 for a tertiary methyl, and a doublet at δ1.71 (J = 1.7 Hz) indicating the presence of a methyl group on an olefinic double bond. An AB quartet centred at δ4.1 with J = 11.5 Hz suggested the presence of methylene protons attached to oxygen. Broad singlets at δ4.70 and 4.78 had the same appearance and belonged to the methylinic protons and another broad singlet at δ5.01 was due to an isolated olefinic proton. Irradiation at δ5.02 collapsed the doublet at δ1.71 but did not affect any other signals. Hence the methyl group at δ1.71 had to be attached to the same olefinic double bond to which the proton at δ5.02 belonged. The methylene protons at C-15 appeared as a doublet of a doublet at δ2.08 (J = 14 Hz) due to geminal coupling. Integration of the protons suggested that the singlet at δ0.90 and the AB quartet at δ4.1 contained three protons and two protons, respectively, whereas the olefinic protons at δ4.70, 4.08 and 5.02 contained protons in the ratio 1:1:4 and integrated for 1.5 protons. This confirmed that the compound was a mixture of two isomers as observed on GC. The ^{13}C NMR data of the mixture are presented in Table 1. The structures of compounds **2a** and **b** were further confirmed by their synthesis from **3** (see below). Thus, all of the above evidence proved that the mixture was made up of the isomeric lactones **2a** and **b**.

The most polar compound, **3**, analysed for $C_{20}H_{30}O_3$, $[M]^+$ *m/z* 318; mp 217–218°; $[\alpha]_D$ − 50°. Its IR spectrum showed the presence of a hydroxyl group (3320–3240 cm^{-1}) and a δ-lactone ring (1730 and 1140 cm^{-1}). The ^1H NMR data showed two tertiary methyls (δ0.87 and 1.33), one downfield due to the presence of a geminal hydroxyl group at C-16. Doublets centred at δ4.05 and 4.21 (J = 14 Hz) accounted for the methylene protons at C-20 which was attached to the lactone oxygen and the doublets of a doublet centred at δ2.08 were due to C-15 methylene protons, typical of kauranoids. The ^{13}C NMR data (Table 1) showed a triplet at δ76.5 due to a methylene carbon bearing the lactone oxygen. Two singlets at δ174.8 and 79.3 were attributed to the δ-lactone carbonyl

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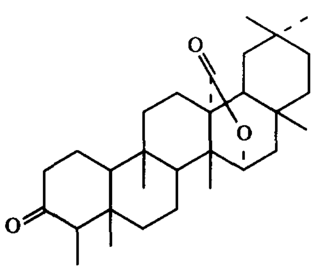
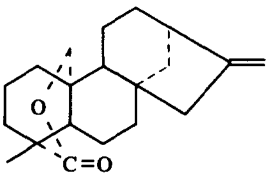
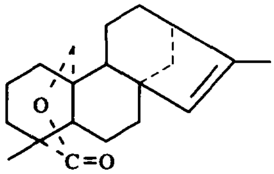


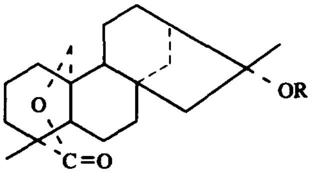
1



2a

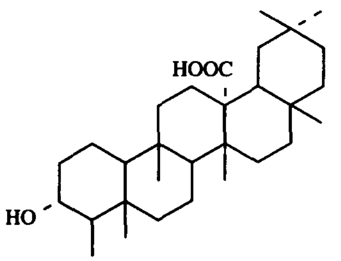


2b



3, R=H

3a, R=Ac



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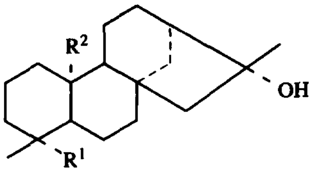

5, R^1 = COOH, R^2 = Me

5a, R^1 = COOMe, R^2 = Me

6, R^1 = CH_2OH, R^2 = CH_2OH

carbon and carbon bearing the hydroxyl group. All the above evidence proved that **3** was 16α-hydroxy-ent-kaura-19 → 20-olide. That lactone **3** had a 16α-hydroxy group, that C-19 was the lactone carbonyl group and it belonged to the ent-kaurane skeleton were unequivocally established by opening the lactone ring by the Li-EDA method [4–6] to give the expected 16α-hydroxy-ent-kaurane-19-oic acid (**5**). Furthermore, dehydration of **3** with $POCl_3$–pyridine gave a mixture that was found to be identical with the mixture of **2a** and **b** isolated from the plant (see above). This confirmed the position of the hydroxyl group as well as the positions of the double bonds in **2e** and **b**.

Acetylation of **3** furnished an acetate **3a**, $[M]^+$ *m/z* 360; IR ν_{max}^{nujol} cm^{-1}: 1730 (δ-lactone), 1725, 1240 (-OAc); ^1H NMR: δ1.98 (3H, *s*, OAc). $LiAlH_4$ reduction of **3** furnished the triol **6**, $C_{20}H_{34}O_3$, $[M]^+$ *m/z* 322; IR: ν_{max}^{nujol} cm^{-1}: 3320–3520 (br, OH). The ^1H NMR spectrum showed the presence of two tertiary methyls (δ1.04 and 1.39), two pairs of methylene protons (δ3.49 and 3.9) as doublets with the same coupling constant ($J = 11$ Hz) suggesting the presence of two methoxyl groupings in **6**.

This is believed to be first report of the isolation of diterpenoids from a member of the Flacourtiaceae. The formation and existence of the C_{19}/C_{20} lactone system in the diterpenoids is a rare occurrence and it is of interest in connection with gibberellin biosynthesis.

EXPERIMENTAL

Mps: uncorr.; IR: nujol; ^1H NMR: 300 MHz, $CDCl_3$ with TMS as int. standard; ^{13}C NMR ($CDCl_3$): 25 MHz; EI-MS: 70 eV; TLC silica gel G developed in I_2 chamber; CC: silica gel 60–120 mesh; petrol: bp 60–80°.

Extraction. Dried and powdered roots (5 kg) of *Gynocardia odorata* were extracted with C_6H_6 in a Soxhlet apparatus for 48 hr. The solvent was distilled off under red. pres. to give a gummy residue (25 g). The residue was separated into neutral and acid parts. The

Table 1. ^{13}C NMR spectral data of compounds **2a, b, 3** and **3a** ($CDCl_3$ as solvent with int. TMS)

C	**2a**	**2b**	**3**	**3a**
1	40.8 *t*	41.0 *t*	40.6 *t*	40.8 *t*
2	20.7 *t*	20.8 *t*	20.5 *t*	20.7 *t*
3	20.4 *t*	22.6 *t*	23.9 *t*	23.4 *t*
4	33.1 *s*	33.1 *s*	32.9 *s*	33.1 *s*
5	50.1 *d*	50.0 *d*	49.7 *d*	49,8 *d*
6	21.5 *t*	21.5 *t*	22.5 *t*	22.7 *t*
7	21.5 *t*	41.5 *t*	39.7 *t*	39.9 *t*
8	43.9 *s*	43.9 *s*	44.7 *s*	44.4 *s*
9	53.1 *d*	53.1 *d*	53.1 *d*	53.1 *d*
10	48.3 *s*	49.0 *s*	47.7 *s*	47.9 *s*
11	19.8 *t*	19.1 *t*	18.8 *t*	19.0 *t*
12	31.0 *t*	31.0 *t*	35.2 *t*	35.3 *t*
13	44.7 *t*	45.7 *t*	49.2 *t*	46.3 *t*
14	39.3 *t*	39.3 *t*	39.1 *t*	39.3 *t*
15	48.2 *t*	132.6 *d*	56.6 *t*	54.3 *t*
16	156.2 *s*	144.10 *s*	79.3 *s*	91.0 *s*
17	102.5 *t*	15.2 *q*	24.3 *q*	19.7 *q*
18	23.8 *q*	23.79 *q*	23.6 *q*	23.8 *q*
19	174.6 *s*	175.15 *s*	174.8 *s*	174.8 *s*
20	76.7 *t*	76.71 *t*	76.5 *t*	76.7 *t*
OCOMe				170.8 *s*
OCOMe				22.7 *q*

neutral gummy residue (20 g) was adsorbed over silica gel (900 g) and the column eluted with solvent mixtures of increasing polarity.

Isolation of odolactone (**1**). The first fraction eluted by C_6H_6–petrol (2:3) was further purified by crystallization from $CHCl_3$–MeOH to afford **1** (0.2 g), mp 307–308°; $[\alpha]_D^{CHCl_3}$ 47°; IR ν^{nujol} cm^{-1}: 1720 ($>$ = 0), 1760 (γ = lactone). Identical (mmp, co-TLC and co-IR) with an authentic sample of odolactone.

Isolation of β-*sitosterol*. The C_6H_6–petrol eluent (3:2) furnished a solid (0.3 g) which on crystallization from $CHCl_3$–MeOH afforded flakes, identified as β-sitosterol by direct comparison with an authentic sample (mmp, co-TLC and co-IR).

Isolation of mixture of odolide (**2a**) *and iso-odolide* (**2b**). The C_6H_6–petrol (4:1) eluate (0.12 g), on crystallization from Me_2CO, furnished needle shaped crystals, mp 131–132°; $[\alpha]_D^{CHCl_3}$ – 72°. It gave a positive TNM test and GC showed it to be an isomeric mixture of two components (**2a** and **b**) in 4:1 ratio. IR ν_{max}^{nujol} cm^{-1}: 1725, 1150 (δ-lactone ring), 3010, 1600, 880 ($C{=}CH_2$), 810 ($>C{=}C<_H$); UV: no absorption above 220 nm; EI-MS (70 eV) *m/z* (rel. int.): 300 $[M]^+$ (100), 285 $[M - Me]^+$ (10), 272 $[M - CO]^+$ (67.6), 257 (53.6), 224 (21.2) 211 (42.1), 183 and 157; ^{1}H NMR: δ0.90 (3H, *s*, Me-18), 1.71 (3H, *d*, *J* = 1.7 Hz, Me-17), 2.08 (2H, *dd*, *J* = 11.5 Hz, H_2-15), 4.1 (2H, AB_q, *J* = 11.5 Hz, H_2-20), 4.78 (2H, 2*br s*, H_2-17), 5.02 (1H, *br s*, H-15). [Found: C, 79.93; H, 9.28. $C_{20}H_{28}O_2$ requires: C, 79.96; H, 9.39%.]

Isolation of 16-*hydroxyl odolide* (**3**). The polar eluent C_6H_6–EtOAc (4:1) afforded a solid which on crystallization from EtOAc-MeOH furnished needle shaped crystals of **3**, mp 217–218°; $[\alpha]_D^{CHCl_3}$ – 50°; IR ν^{nujol} cm^{-1}: 3320–3240 (–OH), 1730, 1140 (δ-lactone); UV: no absorption above 200 nm; EI-MS (70 eV) *m/z* (rel. int.): 318 $[M]^+$ (45), 300 $[M - H_2O]^+$ (12), 285 (22), 272 (25) and 260 (100); ^{1}H NMR: δ0.87 (3H, *s*, Me-18), 1.33 (3H, *s*, Me-17), 2.08 (2H, *dd*, *J* = 11.5 Hz, H_2-15), 4.05 + 4.21 (2H, 2*d*, *J* = 14 Hz, H_2-20). [Found: C, 75.31; H, 10.0. $C_{20}H_{30}O_3$ requires: C, 75.43; H, 9.50%.]

Reduction of compound **3** *with* Li *in ethylenediamine*. To a soln of **3** (0.2 g) in dry ethylenediamine (25 ml), Li metal (0.2 g) was added in small pieces at intervals and the mixture was refluxed for 2 hr under a N_2 blanket. The reaction mixture was cooled and treated with solid NH_4Cl to destroy excess Li metal. It was then acidified with dilute HCl, extracted with Et_2O, washed with H_2O and dried over dry Na_2SO_4. The residue (0.18 g) obtained after removal of Et_2O afforded a solid which on crystallization from $CHCl_3$–MeOH furnished an amorphous solid, **5**, mp 281–282°; $[\alpha]_D^{CHCl_3}$ – 92°. This was esterified with CH_2N_2 and the solid obtained after the usual work-up was crystallized from $CHCl_3$–MeOH to afford the methyl ester **5a**, mp 155–156°; IR ν_{max}^{nujol} cm^{-1}: 3240–3340 (br, –OH), 1720 ($-CO_2Me$); EI-MS (70 eV) *m/z* (rel. int.): 334 $[M]^+$ (12), 316 $[M - H_2O]^+$ (10), 301 $[316 - Me]^+$ (24), 276 (22) and 257 (100); ^{1}H NMR: δ0.74, 0.90 and 1.36 (each 3H, *s*, Me-18, Me-20 and Me-17), 2.06 (2H, *dd*, *J* = 14 Hz, H_2-15), 3.67 (3H, *s*, $-CO_2Me$). [Found: C, 75.31; H, 10.10. $C_{21}H_{34}O_3$ requires: C, 75.41; H, 10.25%.]

Dehydration of compound **3** *with* $POCl_3$–*pyridine*. A mixture of **3** (200 mg), pyridine (4 ml) and $POCl_3$ (2 ml) was heated over a water bath for 6 hr. The residue (0.15 g) obtained after usual work-up was chromatographed over silica gel (10 g). Elution with C_6H_6–petrol (4:1) gave a solid that was crystallized from $CHCl_3$–MeOH to furnish needle shaped crystals, mp 131–132°. IR ν_{max}^{nujol} cm^{-1}: 1725, 1150 (δ-lactone), 3010, 1600, 885 ($C = CH_2$), 800 ($>C{=}\,C<_H$); ^{1}H NMR: almost identical with that of the mixture of **2a** and **b**; no depression in mmp with authentic sample of **2a** and **b** mixture.

Acetylation of compound **3**. Compound **3** (0.2 g) when acetylated with Ac_2O–pyridine afforded **3a** (0.19 g), mp 170°; IR ν_{max}^{nujol} cm^{-1}: 1730 (δ-lactone), 1725, 1240 (–OAc); EI-MS (70 eV) *m/z* (rel. int.): 360 $[M]^+$ (50), 300 $[M\text{-}HOAc]^+$ (100), 285 (10), 272 (30), 257 (80); ^{1}H NMR: δ0.1 (3H, *s*, Me-18), 1.60 (3H, *s*, Me-17), 1.98 (3H, *s*, –OAc), 2.08 (2H, *d*, *J* = 11.5 Hz, H_2-15), 4.04 + 4.24 (2H, 2*d*, *J* = 14 Hz, H_2-20). [Found: C, 73.00; H, 8.20. $C_{22}H_{32}O_4$ requires: C, 72.89; H, 94.5%.]

Reduction of **3** *with* $LiAlH_4$. Compound **3** (0.2 g) in dry Et_2O was refluxed with $LiAlH_4$ (0.2 g) for 8 hr. After usual work-up, the residue (0.15 g) was chromatographed over silica gel (10 g) and elution with MeOH–C_6H_6 (1:49) furnished the triol **6**, mp 199°; IR ν_{max}^{nujol} cm^{-1}: 3320–3520 (b, –OH); EI-MS (70 eV) *m/z* (rel. int.): 322 $[M]^+$ (0.5), 304 $[M - H_2O]^+$ (10), 292 $[M - CHOH]^+$ (17), 286 $[M - 2H_2O]^+$ (16), 274 $[292 - H_2O]^+$ (58), 273 $[M - (CH_2OH + H_2O)]^+$ (100), 255 $[273 - H_2O]^+$ (90), 243 $[M - 2CH_2OH]^+$ (80), 234 (44), 215 (38), 203 (40); ^{1}H NMR: δ1.04 and 1.39 (each 3H, *s*, Me-18 and

Me-17), 2.3 (1H, *d*, $J = 11$ Hz, –OH), 3.49 (2H, *d*, $J = 11$ Hz, H_2-19), 3.9 (2H, *d*, $J = 11$ Hz, H_2-20) and 4.04 (1H, *d*, $J = 11$ Hz, –OH). [Found: C, 74.25; H, 10.20. $C_{20}H_{34}O_3$ requires: C, 74.49, H, 10.63%.]

Isolation of trichadenic acid-A (**4**). The alkali wash on acidification with dilute HCl was extracted with Et_2O. The Et_2O extract was then washed with H_2O and dried (Na_2SO_4). The solvent was evapd and the residue chromatographed over silica gel. Elution of the column with C_6H_6–Et_2O (4:1) gave a solid which on crystallization from $CHCl_3$–MeOH furnished **4** (0.18 g) as needles, mp 292–293°, $[\alpha]_D^{CHCl_3}$ $+25.0°$; IR ν_{max}^{nujol} cm^{-1}: 3300 (OH), 1685 (–CO_2H); identical with an authentic sample of trichadenic acid-A (mmp, co-TLC and co-IR). [Found: C, 78.48; H, 10.80. $C_{30}H_{50}O_3$ requires: C, 78.60; H, 10.92%.]

Acknowledgements—The authors wish to thank Prof. H. Ageta (Showa College of Pharmaceutical Sciences, Tokyo, Japan), Prof. Dr P. Weyerstahl (Technische Universität Berlin), and the Director (CDRI, Lucknow) for some of the spectral data and elemental analysis. One of us (S.C.) wishes to thank CSIR, New Delhi for the award of a Research Associateship.

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