Phytochemistry, Vol. 23, No. 2, pp. 407–410, 1984.
Printed in Great Britain.

0031–9422/84 $3.00 + 0.00

TWO UNSYMMETRIC TETRACYCLIC TRITERPENOIDS FROM *CISSUS QUADRANGULARIS**

K. K. Bhutani,† R. Kapoor and C. K. Atal

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

(*Revised received* 30 *June* 1983)

Key Word Index—*Cissus quadrangularis*; Vitaceae; tetracyclic triterpenes; onocer-7-ene-3α,21β-diol; onocer-7-ene-3β,21α-diol; δ-amyrin; δ-amyrone.

Abstract—Two new unsymmetric tetracyclic triterpenoids onocer-7-ene-3α,21β-diol and onocer-7-ene-3β,21α-diol together with sitosterol, δ-amyrin and δ-amyrone have been isolated from *Cissus quadrangularis*. The structures of the new compounds were elucidated on the basis of ^{1}H NMR, mass spectral and chemical evidence.

INTRODUCTION

The presence of three ketosteroids in *Cissus quadrangularis* has been reported previously but without any structure assignments [2].

The plant stems used for the present study were collected from Maharashtra. The isolation of two new unsymmetric tetracyclic triterpenoids along with sitosterol, δ-amyrin and δ-amyrone from this plant and the spectral and chemical evidence leading to the elucidation of their structures and stereochemistry are discussed.

RESULTS AND DISCUSSION

The ethanolic extract of the plant was taken to dryness and the ether-soluble fraction upon column chromatography on neutral alumina yielded from the benzene eluate fractions δ-amyrone [3] (8.4 % yield), δ-amyrin [3] (12 % yield) and sitosterol (16.8 % yield). The structures of δ-amyrone [3] and δ-amyrin [3] were confirmed by their conversion [4] to β-amyrone and β-amyrin, respectively. The unknown products were compound **1** [mp 200–202°, $[M]^+$ at m/z 444 ($C_{30}H_{52}O_2$); ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1615 (C=C), 1380 (*gem*-dimethyl) and 1055 (hydroxy C–O)] and compound **2** [mp 233–234°, $[M]^+$ at m/z 444 ($C_{30}H_{52}O_2$); ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1615 (C=C), 1380 (*gem*-dimethyl) and 1058 (hydroxy C–O)].

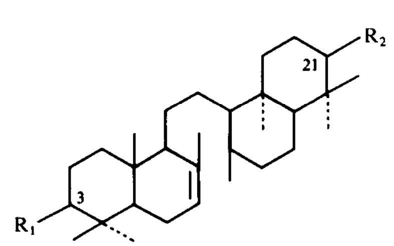
The ^{1}H NMR‡ spectra of **1** and **2** in $CDCl_3$ showed the presence of six tertiary methyl groups on saturated carbons [δ 0.85 (0.83), 0.97 (0.99), 1.02 (1.03), 1.26 (1.11), 1.32 (1.30) and 1.54 (1.55), 3H, *s* each], one secondary methyl on a saturated carbon [δ 0.96 (0.98), 3H, *d*] and one olefinic methyl [δ 1.95 (1.94), *s*], two D_2O-exchangeable protons [δ 1.11 (1.55), *s*] and two deshielded methine protons bearing a hydroxyl group [δ 3.1 (3.1), *s* and 3.67 (3.65), *s*]. Long-range coupling of the olefinic proton at δ 5.01 (5.01), *d* showed that possibly an olefinic methyl was coupled with the olefinic proton.

From the ^{1}H NMR spectrum, it appears that compounds **1** and **2** are similar and differ by the stereochemistry of the hydroxyl groups.

Acetylation (Ac_2O–pyridine) of **1** and **2** gave monoacetylated products **3**, mp 163–164°, and **4**, mp 166–167°. Comparable ^{1}H NMR spectra of derivatives **3** and **4** (one secondary methyl on a saturated carbon 0.96, *d*; one olefinic methyl 2.02, *s*; and olefinic proton 5.01, *d*) confirmed the assignments of a secondary methyl at C-14 and an olefinic methyl at C-8 in the saturated and unsaturated halves of the molecule. Acetylated products **3** and **4** both had $[M]^+$ at m/z 486 ($C_{32}H_{54}O_3$) but differed in their fragmentation patterns (Fig. 1). Both acetylated products gave ions at m/z 453 which must be due to the loss of water by 1,3-elimination involving an α-OH and α-H from the $[M - Me]^+$ fragment [5].

The presence of an onocerane skeleton was demonstrated by the appearance of an olefinic methyl at δ 1.95 (1.94) and an olefinic proton at 5.01 (5.01) and their coupling was, as observed earlier, at δ 1.68 (3H) and 5.44 (1H) for the structure elucidation of the first reported unsymmetric onoceradiene dione [6].

The nature of the carbon skeleton of the unsymmetric onocerene structures **1** and **2** and their derivatives **3** and **4** was further revealed by the mass spectral fragmentation



1 R_1 = α–OH; R_2 = β–OH
2 R_1 = β–OH; R_2 = α–OH
3 R_1 = α–OH; R_2 = β–OAc
4 R_1 = β–OAc; R_2 = α–OH
5 R_1 = R_2 = O

*Part II in the series "Investigations of Medicinal Plants". For Part I see ref. [1].

†To whom correspondence should be addressed.

‡Data for compound **2** given in parentheses.

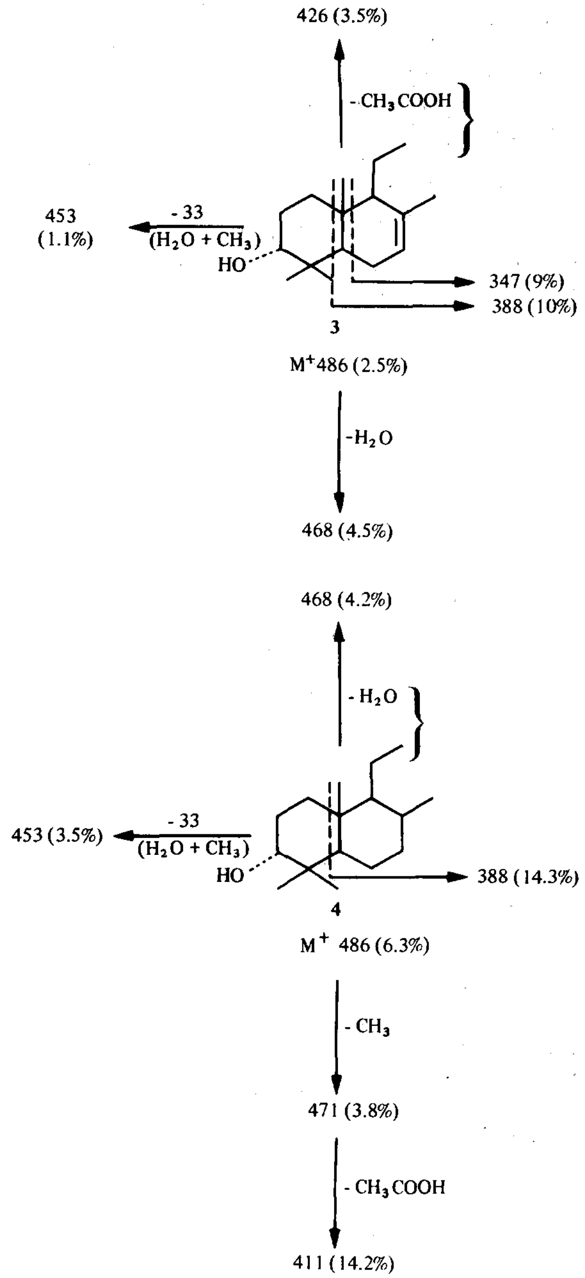


Fig. 1.

behaviour of these compounds. The significant ions with appropriate mass shifts in their mass spectra, characteristic of onocerane [6], are shown in Fig. 2. The ions at m/z 205 (67.2 %) and 191 (31.8 %) in the mass spectra of **1** and **2** are due to C-11/C-12 bond cleavage [5] and C-9/C-11 bond cleavage, respectively. The mass spectral fragments at m/z 151 (33.2 %), 137 (55.2 %) and 205 (67.2 %), 123 (52.4 %), 109 (88 %) and 95 (100 %) are obtained from (c) and (d) cleavage of compounds **1** and **2**.

The different stereochemistries of the hydroxyl groups at C-3 and C-21, for compounds **1** and **2** were well illustrated by comparison of the mass shifts of the appropriate ions at m/z 486 $[M]^+$ (2.5 %), 468 (4.5 %), 453 (1.1 %), 426 (3.5 %), 388 (10 %), 347 (9 %) and 486 $[M]^+$ (6.3 %), 471 (3.8 %), 468 (4.2 %), 453 (3.5 %), 411 (14.2 %), 388 (14.3 %) of the monoacetylated products **3** and **4**, respectively. The other significant ions listed in the Experimental arose following the fragmentation pathway given for onocer-7-ene. The fragmentation patterns of acetylated products **3** and **4** clearly demonstrated that the position of the axial hydroxyl group which resisted acetylation varied in the two unsymmetrical halves of the molecule. In compound **3** the axial hydroxyl group was in the unsaturated half and in compound **4** it was in the saturated half of the molecule.

From the above data, the structures of two new tetracyclic triterpenoids are deduced as **1** and **2**. The relationship between structures **1** and **2** was further confirmed by Jones oxidation [7], which gave the same dioxo derivative, **5**, mp 128–131° (confirmed by mixed TLC, mmp and superimposable IR).

EXPERIMENTAL

Mps are uncorr. ^1H NMR δ values in ppm downfield from TMS. TLC spots developed by 2 % cerric sulphate soln in H_2SO_4 and heating at 110°.

Extraction. Air-dried whole plant (1 kg) of *Cissus quadrangulariswas* extracted exhaustively by cold percolation with EtOH. The extract was dried under red. pres. and the Et_2O-soluble portion (12.5 g) was chromatographed over neutral Al_2O_3 using solvent and solvent mixtures of increasing polarity. The similar fractions as indicated by TLC were combined.

Isolation of δ-amyrone [olean-13(18)-en-3-one]. The first fractions (4 × 100 ml) of the C_6H_6 eluate from the Al_2O_3 chromatography afforded a solid (1.3 g) showing a single spot on TLC (hexane–EtOAc, 4:1), crystallized from EtOAc as colourless flakes (1.0 g; 8.4 % yield); mp 176–178°; IR ν_{max}^{KBr} cm^{-1}: 2930, 2865, 1710, 1450, 1380, 1140, and 885; MS m/z (rel. int.): 424 $[M]^+$ (70), 409 (38), 218 (40), 205 (100), 189 (50) (characteristic of olenane skeleton); $C_{30}H_{48}O$ calc. from high resolution. ^1H NMR ($CDCl_3$): δ 0.73 (*s*, 3H), 0.93 (*s*, 3H), 1.00 (*s*, 3H), 1.07 (*s*, 3H), 1.22 (*s*, 3H), 1.40 (*s*, 3H), 1.48 (*s*, 6H). The structure was confirmed by conversion to β-amyrone [3] and comparison with an authentic sample of β-amyrone (mp, mmp, TLC and superimposable IR).

Isolation of δ-amyrin [olean-13(18)-en-3-ol]. The next fractions (5 × 100 ml) of the Al_2O_3 chromatography afforded a solid (1.8 g) showing a single spot on TLC (hexane–EtOAc, 4:1), crystallized from EtOAc as colourless crystals (1.4 g, 12 % yield), mp 156–158°; IR ν_{max}^{KBr} cm^{-1}: 3440, 2930, 1635, 1455, 1380, 1035; MS m/z (rel. int.): 426 $[M]^+$ (95), 411 (78), 218 (72), 205 (100), 189 (57) (characteristic of olenane skeleton); $C_{30}H_{50}O$, calc. from high resolution. ^1H NMR ($CDCl_3$): δ0.63 (*s*, 3H), 0.66 (*s*, 3H), 0.82 (*s*, 3H), 0.97 (*s*, 3H), 1.0 (*s*, 3H), 1.06 (*s*, 1H, D_2O exchangeable), 1.25 (*s*, 3H), 1.34 (*s*, 3H), 1.48 (*s*, 3H), 3.25 (*m*, 1H). The structure was confirmed by conversion to β-amyrin [4] and comparison with an authentic sample of β-amyrin (mp, mmp, TLC and superimposable IR).

The later fractions yielded sitosterol, confirmed by direct comparison (mp, mmp, TLC and superimposable IR) with an authentic sample.

Isolation of onocer-7-ene-3α,21β-diol (**1**). The $CHCl_3$ eluate of the Al_2O_3 chromatography afforded a gummy mass (700 mg) showing two close spots on TLC ($CHCl_3$–MeOH, 19:1). Upon repeated chromatography over Al_2O_3 this yielded 100 mg of material showing a single spot in the same TLC system. Crystallized from MeOH as colourless crystals (60 mg, 0.048 % yield), mp 200–202°; IR ν_{max}^{KBr} cm^{-1}: 3350, 1615, 1380, 1055. MS

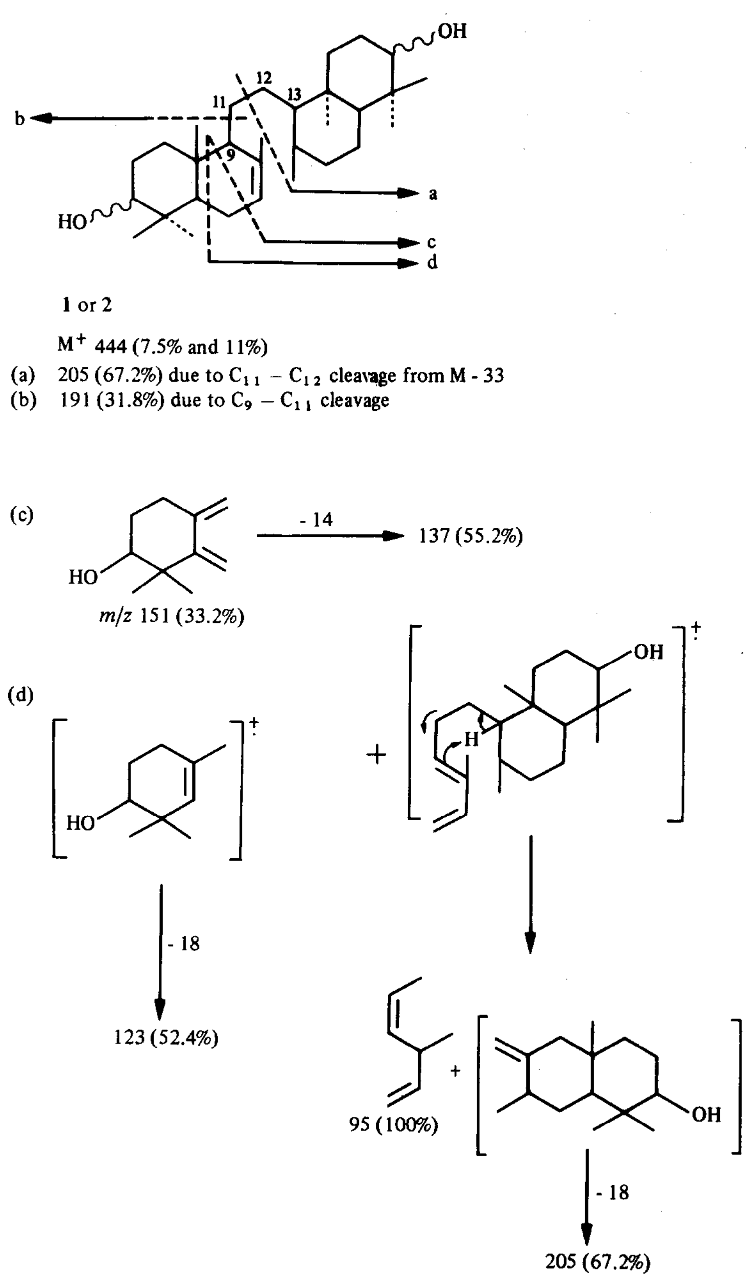


Fig. 2.

m/z (rel. int.): 444 $[M]^+$ (7.5), 411 (20.5), 347 (21.6), 317 (7.7), 205 (67.2), 191 (31.8), 151 (33.2), 137 (55.2), 123 (52.4), 109 (88), 95 (100) (characteristic of onocerane skeleton); $C_{30}H_{52}O_2$, calc. from high resolution. ^1H NMR ($CDCl_3$): δ 0.85, 0.97, 1.02, 1.26, 1.32 and 1.54 (3H, *s* each), 0.96 (3H, *d*), 1.95 (3H, *s*), 1.11 (2H, *s*, D_2O exchangeable), 3.1 (1H, *s*), 3.67 (1H, *s*), 5.01 (1H, *d*).

Isolation of onocer-7-ene-3β,21α-diol (**2**). Further $CHCl_3$ eluates after recovery of **1** afforded 30 mg (0.024% yield) of solid **2** showing a single spot on TLC ($CHCl_3$–MeOH, 19:1) crystallized with MeOH, mp 233–234°; IR ν_{max}^{KBr} cm^{-1}: 3350, 1615, 1380, 1058. MS *m/z* (rel. int.): 444 $[M]^+$ (11), 427 (11), 411 (2), 385 (4), 347 (22), 317 (8), 205 (67.2), 191 (31.8), 151 (33.2), 137 (55.2), 123 (52.4), 109 (88.0), 95 (100) (characteristic of onocerane skeleton). $C_{30}H_{52}O_2$, calc. from high resolution. ^1H NMR ($CDCl_3$): δ 0.83, 0.99, 1.03, 1.11, 1.30, 1.55, (3H, *s*) each, 0.98 (3H, *d*), 1.94 (3H, *s*), 1.55 (2H, *s*, D_2O exchangeable), 3.1 (1H, *s*), 3.65 (1H, *s*) and 5.01 (1H, *d*).

Onocer-7-ene-3α,21β-diol 21-acetate (**3**). A soln of **1** (25 mg) in pyridine was treated with Ac_2O (2 ml) (room temp. 18 hr). The residue obtained after usual work-up afforded a monoacetate (30 mg) (**3**), crystallized from MeOH as colourless crystals (20 mg), mp 163–164°; MS *m/z* (rel. int.): 486 $[M]^+$ (2.5), 468 (4.5), 453 (1.1), 426 (3.5), 388 (10), 347 (9), 317 (6.5), 205 (87.9), 191 (36.8), 151 (42.8), 95 (100). ^1H NMR ($CDCl_3$): δ 2.02 (3H, *s*), 1.25 (1H, *s*, D_2O exchangeable), 3.1 (1H, *s*), 4.55 (1H, *s*) and 5.01 (1H, *d*).

Onocer-7-ene-3β,21α-diol 3-acetate (**4**). A soln of **2** (15 mg) in pyridine was treated with Ac_2O (2 ml) (room temp. 18 hr). The residue obtained after usual work-up afforded the monoacetate (17 mg) (**4**), crystallized from MeOH as colourless crystals, mp 166–167°. MS *m/z* (rel. int.): 486 $[M]^+$ (6.3), 471 (3.8), 468 (4.2), 453 (3.5), 411 (14.2), 388 (14.3), 317 (8.4), 251 (11.1), 205 (70.5), 191 (27.9), 151 (37.3), 95 (100). ^{1}H NMR ($CDCl_3$): δ 2.02 (3H, *s*), 1.25 (1H, *s*, D_2O exchangeable), 3.1 (1H, *s*), 4.55 (1H, *s*), 5,01 (1H, *d*).

Onocer-7-ene-3,21-dione (**5**). Jones reagent [7] (2 drops) was added to a cold soln (10–15°) of compounds **1** and **2** (10 mg, each) in Me_2CO (5 ml). After 2 min the reaction mixture was diluted with H_2O (20 ml) and extracted with $CHCl_3$ (3 × 20 ml). The $CHCl_3$ extract was washed, dried and solvent removed under red. pres. to obtain residue **5** (not crystallizable), mp 128–131°. Both compounds **1** and **2** gave the same product having the same mmp, TLC and superimposable IR. IR ν_{max}^{KBr} cm^{-1}: 2985, 1715, 1705, 1615, 1380.

Acknowledgements—We are grateful to the instrumental division of our laboratory for providing spectral data of compounds. Thanks are also due to the Hoechst Laboratory for providing plant material.

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