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A NEW LUPENE DIOL FROM *DODONAEA ATTENUATA*

Emilio L. Ghisalberti, Philip R. Jefferies and Mark A. Sefton

Department of Organic Chemistry, University of Western Australia, Nedlands, 6009, Australia

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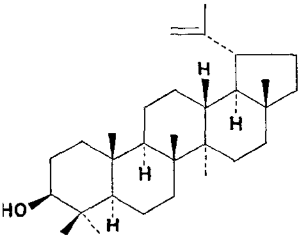
Key Word Index—*Dodonaea attenuata*; Sapindaceae; lupene diol; lupeol; triterpenes.

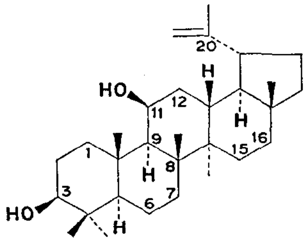
Abstract—Lup-20(29)-ene-3β,11β-diol and lupeol have been isolated from *Dodonaea attenuata* A. Cunn. var. *linearis* Benth.

INTRODUCTION

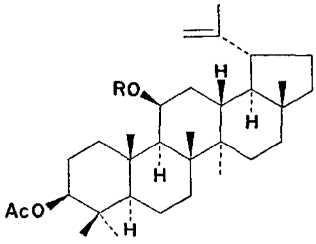
Dodonaea attenuata A. Cunn. var. *linearis* Benth. (Sapindaceae) is a viscid shrub which grows to a height of 2 m and can be distinguished by its long narrow leaves. An examination of the acidic constituents resulted in the isolation of two furanoditerpenes[1] and a flavone.[2]



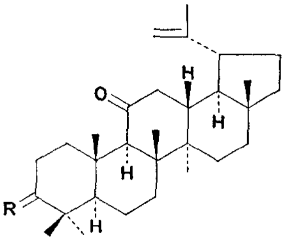
(I)



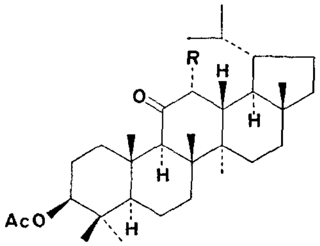
(II)



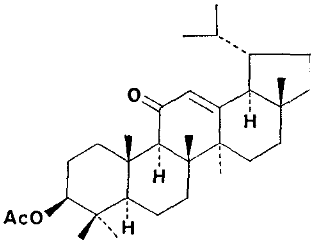
(III) R = H
(IV) R = Ac



(V) R = $\begin{matrix}\blacktriangleleft\text{OAc}\\ \cdots\text{H}\end{matrix}$
(VI) R = $\begin{matrix}\blacktriangleleft\text{OH}\\ \cdots\text{H}\end{matrix}$
(VII) R = O



(VIII) R = H
(IX) R = Br



(X)

Investigation of the neutral fraction has now led to the isolation of lupeol (I) and a new triterpene diol, lup-20(29)-ene-3β,11β-diol (II), the subject of this communication. The presence of the two triterpenes was initially detected by noting the incorporation of [2-^{14}C]-mevalonic acid (MVA) fed to the plant into the neutral constituents. The biosynthesis of

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[1] Jefferies, P. R. and Payne, T. G. (1967) *Tetrahedron Letters* 4777.

[2] Jefferies, P. R. and Payne, T. G., unpublished results.

steroids and triterpenoids from MVA is now well established and appears to hold for all living organisms.[3] When radioactive MVA was applied to *D. attenuata* var. *linearis* seedlings the major portion of the radioactivity incorporated was found in the neutral fraction.[4] A closer investigation showed that the radioactivity was associated with lupeol (I), the diol (II), a 4-desmethyl sterol fraction and unidentified compounds present in trace amount. Lupeol and the diol (II) were obtained in greater quantity from an unlabelled extract of the plant.

Surprisingly the use of radioactive MVA as a marker for the detection of steroid and triterpene constituents of plants has not been widely employed. This method appears to have some potential in the initial detection of these compounds which, if present in minor quantities, may otherwise be overlooked.

RESULTS AND DISCUSSION

Chromatography of the neutral fraction from an extract of *D. attenuata* var. *linearis* afforded, (a) lupeol, (b) a sterol mixture, and (c) a new triterpene (II), $C_{30}H_{50}O_2$, m.p. 187–188° which was characterized by formation of a monoacetate (III) and a diacetate (IV), thus identifying it as a diol. The NMR spectrum (90 MHz) of II showed signals for six tertiary methyls (δ 0·79, 0·84, 0·94, 0·96, 1·32 and 1·37) and a vinylic methyl (δ 1·68), which was shown to be coupled to two vinylic protons at δ_A 4·58; δ_B 4·71 (J_{AB} 2·5 Hz) indicating the presence of an isopropenylidine group. Resonance signals for two carbinol methine protons appeared at δ 3·18 (3-H; $W_{1/2}$ 18 Hz) and 4·24 (11-H, $W_{1/2}$ 9 Hz) being shifted to δ 4·65 and 5·35 respectively in the NMR spectrum of the diacetate (IV). Jones' oxidation of the monoacetate (III) yielded the ketoacetate (V) which on reduction under forcing Wolff–Kishner conditions[5] afforded lupeol (I) and the hydroxyketone (VI). This result established the nature of the diol as a hydroxylated derivative of lupeol. The location of the second hydroxyl group was limited to position 6, 7, 11 or 15 by the following considerations. Absorption maxima in the IR and UV spectra of the diketone (VII) (ν_{max} 1705 cm^{-1}; λ_{max} 290; ϵ 110, unchanged on addition of base) excluded the second hydroxyl group from the *E*-ring or a 1,2- or 1,3-relationship with the 3-C hydroxyl of lupeol. Furthermore both 1α, 3β- and 1β,3β-dihydroxylup-20(29)-ene have been reported[6] and their properties differ from those of the diol.

While only one epimer (12β-) of 3β,12-dihydroxylup-20(29)-ene is known,[7] the difference in the physical properties of the derived 3,12-diketone and the diketone (VII) are sufficient to exclude C-12 as the site for the second hydroxyl in the diol (II). Position 16 can similarly be excluded by comparison of the dihydroketoacetate (VIII) with the known 3β-acetoxylupan-16-one.[8] A choice between C-6, C-7, C-11 and C-15 was indicated by analysis of the NMR data obtained for the diol (II) and the monoacetate (III). The axial configuration of the second hydroxyl group was evident from the NMR spectra of (II) and (III) which showed the resonance signal for the carbinol methine proton at δ 4·24 ($W_{1/2}$ 9 Hz) and

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[3] GOODWIN, T. W. (1971) *Biochem. J.* **123**, 293.

[4] KNOX, J. R. and PRICE, S. H., unpublished results. We are indebted to Dr. Knox for providing us with the neutral extract from this experiment.

[5] ITAZAKI, H. and NAGATA, W. (1964) *Chem. Ind.* (*London*) 1194.

[6] GANGULY, A. K., GOVINDACHARI, I. R., MOHAMED, P. A., RAHIMTULLA, A. D. and VISWANATHAN, N. (1966) *Tetrahedron* **22**, 1513; HUI, W. H. and FUNG, M. L. (1969) *J. Chem. Soc.* C, 1710.

[7] JOLAD, S. D. and STEELINK, C. (1969) *J. Org. Chem.* **44**, 1367.

[8] BADDELEY, G. V., BEALING, A. J., JEFFERIES, P. R. and RETALLACK, R. W. (1964) *Australian J. Chem.* **17**, 908.

4·30 ($W_{1/2}$ 10 Hz) respectively, consistent with an equatorial rather than an axial proton. Whereas in the NMR spectrum of lupeol (I) the chemical shifts of the tertiary methyl protons appear between δ 0·75 and 1·05, the NMR spectrum of the diol (II) shows signals for four methyl groups between δ 0·79 and 0·96, the other two appearing at δ 1·32 and 1·37. Thus the introduction of the second hydroxyl group has resulted in the deshielding of two methyl groups by at least 0·27 ppm. Such an effect is best interpreted in terms of 1,3-diaxial interactions between the hydroxyl and the two methyl groups. This is confirmed by a comparison of the NMR spectra of the monoacetate (III) in pyridine and chloroform which shows that in pyridine the two methyl groups are further deshielded (0·26 ppm) to the extent that might be anticipated for such interactions (0·25–0·32 ppm).[9]

Such considerations militate against position C-6 and C-7 since in these positions an axial hydroxyl would be expected to deshield three and one methyl groups respectively. Also, $LiAlH_4$ reduction of the ketoacetate (V) regenerated the diol (II) whereas reduction of a C-7 carbonyl by metal hydride would lead to a mixture of epimeric alcohols.[10] To choose between position 11 and 15 the following sequence was undertaken: treatment of the dihydroketoacetate (VIII) with Br_2 under acidic conditions gave the bromoketone (IX) the NMR spectrum of which showed a doublet at δ 4·18 (J 3·5 Hz), attributed to an equatorial bromomethine proton,[11] and a one proton singlet at δ 3·33. These observations can best be explained by assuming the presence of a system $-C(H)(H_C)-CO-C(H_B)(Br)-C(H_A)(H)-$ in which H_C and the bromine group are in a 1,3-diaxial relationship.[12] Dehydrobromination of IX with $LiCl-Li_2CO_3$ yielded the enone (X; $\nu_{max}^{CCl_4}$ 1735, 1670 cm^{-1}; λ_{max}^{EtOH} 246 nm; ϵ 9000). These results can only be reconciled with the presence of a C-11 carbonyl in the dihydrodiketo acetate (VIII) but not for a carbonyl at C-6, C-7, or C-15. The triterpene diol is thus assigned the structure of lup-20(29)-end-3β,11β-diol (II).

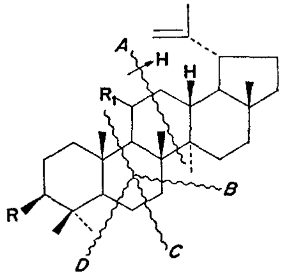

FIG. 1. MS FRAGMENTATION PATTERNS FOR II AND DERIVATIVES.

The occurrence of an 11-hydroxylated lupeol does not appear to have been reported previously although the isolation of 11-keto *epi*-lupeol is noted.[13] However, the data reported for the corresponding diketone (m.p. 178–180°; $[\alpha]_D$ $+71·6°$ are in contrast with

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[9] Demarco, P. V., Doddrell, D., Farkas, E., Mylari, B. L. and Wenkert, E. (1968) *J. Am. Chem. Soc.* **90**, 5480; Ricca, S., Rindone, B. and Scolastico, C. (1969) *Gazz. Chim. Ital.* **99**, 1284.

[10] Corbett, R. E. and Young, H. (1966) *J. Chem. Soc.* **C**, 1556.

[11] Baretta, A., Zahra, J. P., Waegell, B. and Jefford, C. W. (1970) *Tetrahedron* **26**, 15.

[12] Bhacca, N. S. and Williams, D. H. (1964) *Applications of NMR Spectroscopy in Organic Chemistry*, 183 pp., Holden–Day, San Francisco.

[13] Estrada, H., Estrada, E. and Maya, L. (1965) *Bol. Inst. Quim. Univ. Nacl. Auton. Mex.* **17**, 68; *idem.* (1966) *Chem. Abstr.* **64**, 3617c.

those for VII (m.p. 262°; $[\alpha]_D$ +19°). On the basis of our results the structure assigned to the triterpene from *Fluorensia resinosa* must be regarded as doubtful.

The MS fragmentations observed for II and its derivatives were consistent with the structure proposed. The base peak in the MS of the diol (II) and the monoacetate (III) appears at *m/e* 204 and probably arises by fragmentation of the 11-, 12- and 8-, 14-bonds, the positive charge remaining on the fragment containing the *D*- and *E*-rings (Fig. 1). Also significant and common to most derivatives is the ion arising by fragmentation of the same bonds but with concurrent hydrogen transfer (Fragmentation *A*), the positive charge now being retained on the other half of the molecule. All derivatives with an 11-carbonyl group gave rise to ions arising from initial fission of the 9,10-bond followed by fission of the 7,8-, 6,7- and 5,6-bonds respectively (*B*, *C* and *D*) with a concomitant hydrogen transfer to the charged fragment. The MS of the enone (X) shows the base peak at *m/e* 233, the ion arising from fragmentation of the 9,11- and 8,14-bonds with hydrogen transfer, and a significant peak at *m/e* 273 arising from fragmentation of type-B.

EXPERIMENTAL

General experimental details are as described previously.[14] MS were recorded with a Varian Mat CH7 Spectrometer, operating at 70 eV unless otherwise stated. NMR spectra were recorded on a Varian A60 Spectrometer for $CHCl_3$ or $CDCl_3$ solutions, unless otherwise stated, and a Bruker Spectrospin High Resolution NMR spectrometer (90 MHz).

Isolation of the neutral constituents of D. attenuata *var.* linearis. The neutral extract (100 g) of the plant (1·5 kg) was saponified by refluxing for 1 hr with 5% NaOH in aq. EtOH. The residue (30 g), isolated with Et_2O, was adsorbed onto a column of neutral Alumina Act. III (700 g) and eluted with a polarity gradient of light petrol.–Et_2O (50:1 to 3:1; fractions 1–24) followed by Et_2O (fractions 25–31) and MeOH (fractions 32–38). Fraction 24 (3·24 g) was rechromatographed as above to yield a crystalline solid (2·35 g) which recrystallized from acetone as needles of lupeol (I), m.p. and m.m.p. 214–215°, identical with an authentic sample. The combined fractions 25–27 (8·2 g) were rechromatographed to yield a sterol fraction (1·26 g) and a fraction (700 mg) which crystallized from aq. MeOH as needles of the *diol* (II), m.p. 187°, $[\alpha]_D$ + 65° (Found: C, 81·5; H, 11·3. $C_{30}H_{50}O_2$ requires: C, 81·4; H, 11·4%.) ν_{max} 3600 (OH), 880 cm^{-1} (isopropylene double bond). MS. *m/e* (%) 442(20), 424(70), 237(60), 204(100), 191(90), 189(80), 175(70). NMR (90 MHz) δ, 0·79, 0·84, 0·94, 0·96, 1·32, 1·37 (*s*, tertiary methyls), 1·68 (*s*, 20–Me), 3·18 (*m*, $W_{1/2}$ 18 Hz, 3α-H), 4·24 (*m*, $W_{1/2}$ 9 Hz, 11α-H), 4·58 and 4·71 (*m*, J_{AB} 2·5 Hz, 29-H_2).

3β-*Acetoxylup*-20(29)-*en*-11β-*ol* (III). The *monoacetate* was prepared from II with Ac_2O–pyridine and recrystallized from MeOH as needles, m.p. 240–243°. (Found: C, 79·3; H, 10·7. $C_{32}H_{52}O_3$ requires: C, 79·3; H, 10·8%.) ν_{max} 3600 (OH), 1735, 1235 cm^{-1} (acetate). MS: *m/e* (%) 484(20), 466(80), 279(50), 204(100), 191(90), 189(95), 175(70). NMR δ: ($CHCl_3$) 0·84(9H), 0·94(3H), 1·37(6H) (*s*, tertiary methyl groups), 2·04 (*s*, $-OCO\underline{CH}_3$), 4·30 (*m*, $W_{1/2}$ 10 Hz, 11α-H) and 4·65 (*m*, vinyl protons and 3α–H). NMR δ (pyridine): 0·87, 0·91, 0·95, 1·03, 1·63, 1·61 (*s*, tertiary methyls).

3β,11β-*Diacetoxylup*-29(29)-*ene* (IV). The diol (II; 50 mg) NaOAc (100 mg) and Ac_2O (5 ml) were refluxed for 1·5 hr. The product (48 mg) recovered with ether recrystallized from acetone as plates of the *diacetate* (IV) m.p. 192–198°, $[\alpha]_D$ +93°. (Found: C, 77·7; H, 10·4. $C_{34}H_{54}O_4$ requires: C, 77·5; H, 10·3%.) ν_{max} 1735, 1230 cm^{-1} (acetate) MS (20 eV): *m/e* (%) 526(2·5), 466(100), 215(50), 202(85). NMR δ: 0·83(6H), 0·95, 1·08, 1·25, 1·35 (*s*, tertiary methyls) 2·04(6H, *s*, 2 × $-OCO\underline{CH}_3$) 4·65 (*m*, vinyl protons and 3α–H), 5·35 (*m*, $W_{1/2}$ 10 Hz, 11α-H).

3β-*Acetoxylup*-20(29)-*en*-11-*one* (V). The monoacetate (III; 150 mg) in acetone (20 ml) was oxidized with excess Jones' reagent. The product (138 mg) recovered with ether recrystallized from cyclohexane as plates of the *ketoacetate* (V), m.p. 255–258° (decomp.), $[\alpha]_D$ +98°. (Found: C, 79·5; H, 10·3. $C_{32}H_{50}O_3$ requires: C, 79·6; H, 10·4%.) ν_{max} 1735, 1235 (acetate), 1705 cm^{-1} (cyclohexanone). MS: *m/e* (%) 482(100), 299(45), 286(35), 277(85), 273(20), 191(60), 189(75), 175(60). The NMR spectrum included signals at δ: 1·70 (*s*, 20–Me), 2·05 (*s*, $-OCO\underline{CH}_3$), 4·53 (*m*, 3α-H) 4·72 (*m*, vinyl protons).

Reduction of 3β-*acetoxylup*-20(29)-*en*-11-*one* (V). (a) The ketoacetate (V; 146 mg), hydrazine hydrate (1 g) and hydrazine dihydrochloride (250 mg) in diethylene glycol (4·5 ml) were heated at 150° under N_2 for 12 hr. Powdered KOH (380 mg) was added and the temperature raised to 240°, allowing the low boiling material to distil, and held at this temp. for 3 hr. Recovery of the reaction products with Et_2O afforded a mixture of two components which were separated by preparative TLC. The less polar component recrystal-

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[14] BAKKER, H. J., GHISALBERTI, E. L. and JEFFERIES, P. R. (1972) *Phytochemistry* **11**, 2221.

lized from acetone as needles m.p. 214–215°, and was identical (TLC, m.m.p., MS) with an authentic sample of lupeol (I). The more polar component was identified as the ketol (VI), ν_{max}3600 (OH), 1705 cm^{-1} (cyclohexanone) MS: *m/e* (%) 440(100), 299(35), 286(25), 273(15), 235(70), 207(20), 191(40). (b) the ketoacetate (V; 20 mg) in Et_2O (10 ml) was treated with $LiAlH_4$ (100 mg) at reflux temp. for 0·5 hr. Recovery of the product gave a residue (19 mg) which was recrystallized from aq. MeOH as needles, m.p. 187°, unchanged on admixture with a sample of the diol (II).

Lup-20(29)-*ene*-3,11-*dione* (VII). The diol (II; 50 mg) in acetone (20 ml) was treated with excess Jones' reagent for 0·5 hr. The product recovered with Et_2O was purified by preparative TLC to give a fraction (42 mg) which was recrystallized from C_6H_6 as needles of the *diketone* (VII), m.p. 262° (decomp.), $[\alpha]_D$ +19°. (Found: C, 81·8; H, 10·7. $C_{30}H_{46}O_2$ requires: C, 82·1; H, 10·6%.) ν_{max} 1705 cm^{-1} (cyclohexanone), λ_{max} 290 nm (ϵ 110). MS. *m/e* (%) 438(100), 299(30), 286(10), 273(9), 233(55), 207(25), 189(50). NMR δ: 0·80, 1·06(9H), 1·19, 1·38 (*s*, tertiary methyls) 1·70 (*s*, 20-Me), 4·72 (*m*, $W_{1/2}$ 14 Hz, 29-H_2).

3β-*Acetoxylupan*-11-*one* (VIII). The ketoacetate (V; 100 mg) was hydrogenated in EtOH (50 ml) over platinum for 5 hr. The *dihydroketo acetate* (VIII) recrystallized from cyclohexane as plates, m.p. 291–294° (decomp.), $[\alpha]_D$ −13°. (Found: C, 79·3; H, 10·8. $C_{32}H_{52}O_3$ requires: C, 79·3; H, 10·8%.) ν_{max} 1735 (acetate), 1705 cm^{-1} (cyclohexanone). MS (40 eV): *m/e* (%) 484(100), 424(10), 301(10), 288(20), 277(25), 275(6). NMR δ: 0·77, 0·86, 1·02, 1·16, 1·23 (*s*, *tertiary methyls*) 2·03 (*s*, $-OCOC\underline{H}_3$), 4·48 (*m*, $W_{1/2}$ 18 Hz, 3α-H).

3β-*Acetoxylup*-12-*en*-11-*one* (X). The dihydroketo acetate (VIII; 50 mg), Br_2 (0·5 ml) and 60% HBr (1 drop) in AcOH (3 ml) were heated at 100° for 20 min. Recovery of the product with Et_2O and preparative TLC afforded a mixture (30 mg) of VIII and the bromoketone (IX) which could not be separated. The MS of the mixture included peaks at *m/e* (%) 564(9), 562(10), 483(80) attributed to IX and 484, 301, 288 attributed to VIII. The NMR spectrum of the mixture indicated that the bromoketone was by far the major product, resonances attributable to VIII not being detectable. NMR δ: 0·79, 0·88(9H), 1·28, 1·35 (*s*, tertiary methyls), 2·07 (*s*, $-OCOC\underline{H}_3$), 3·35 (*s*, 9-H), 4·18 (*d*, *J* 3·5, 12β-H). 4·56 (*m*, $W_{1/2}$ 18 Hz, 3α-H). ν_{max} 1705 (cyclohexanone), 1735 cm^{-1} (acetate). The above mixture (52 mg), LiCl (60 mg) and Li_2CO_3 (15 mg) in DMF (5 ml) was heated with stirring at 140° for 5 hr. The residue isolated with Et_2O consisted, from TLC analysis, of a separable mixture of VIII and the enone (XI). Preparative TLC afforded VIII as the less polar component, the more polar component was identified as the *enone* (XI; 17 mg) which was recrystallized from MeOH–Et_2O as prisms, m.p. 260–261°, $[\alpha]_D$ +21°. (Found: 482·37597. $C_{32}H_{50}O_3$ requires: 482·37605.) ν_{max} 1735, 1235 (acetate), 1665 (α,β-unsaturated cyclohexanone), 785 cm^{-1} (trisubstituted olefin). λ_{max} 246 nm (ϵ 9000). MS: *m/e* (%) 482(10), 422(4), 273(100), 233(10). NMR δ: 0·66, 0·86(6H), 1·12, 1·20, 1·28 (*s*, tertiary methyls), 1·98 (*s*, $-OCOC\underline{H}_3$), 4·37 (*m*, $W_{1/2}$ 18 Hz, 3α-H), 5·38 (*s*, 12-H).