Phytochemistry, Vol. 25, No. 7, pp. 1745–1747, 1986.
Printed in Great Britain.

0031-9422/86 \$3.00 + 0.00
Pergamon Journals Ltd.

(+)-EUDESM-3-ENE-6β,7α-DIOL FROM THE LIVERWORT *LEPIDOZIA REPTANS*

JOSEPH D. CONNOLLY, LESLIE J. HARRISON, SIEGFRIED HUNECK* and DAVID S. RYCROFT

Department of Chemistry, University of Glasgow, Glasgow G 12 8QQ, Scotland, U.K.; *Institute of Plant Biochemistry of the Academy of Sciences of the GDR, GDR-4020 Halle/Saale, G.D.R.

(*Received* 15 *November* 1985)

Key Word Index—*Lepidozia reptans*; Jungermanniales; Hepaticae; sesquiterpene; eudesm-3-ene-6β,7α-diol.

Abstract—The structure of a new sesquiterpene diol from the liverwort *Lepidozia reptans* has been established as eudesm-3-ene-6β,7α-diol on the basis of its ^{1}H and ^{13}C NMR spectroscopic properties.

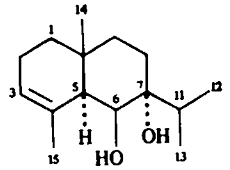
INTRODUCTION

Lepidozia reptans L. Dum. of the family Lepidoziaceae is a rather common liverwort in Europe and grows on rotten wood and silicaceous rocks preferably in the mountains. The leaves contain 10–15 colourless, globular, oval or rodlike oil bodies per cell which give the plant a typical smell. The following sesquiterpenes have been described from *L. reptans*: α- and β-gymnomitrene [1, 2], α-longipinene [1], bicyclogermacrene, β-cubebene, cuparene and β- and δ-elemenene. We have analysed *L. reptans* once again and describe the isolation of the new sesquiterpene eudesm-3-ene-6β,7α-diol (**1**).

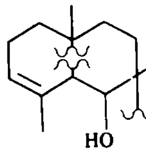
RESULTS AND DISCUSSION

Eudesm-3-ene-6β,7α-diol, $C_{15}H_{26}O_2$, from a sample of *L. reptans* collected in the Harz Mountains (GDR) has the mp 94–95° and $[\alpha]_D^{24}$ + 34.6 and in its ^{1}H NMR spectrum (see Scheme 1) resonances for one tertiary methyl group [δ_H 1.02 (*t*, $J = 0.7$ Hz, 3H-14)], two secondary methyl groups [δ_H 0.95 and 0.92 (2 × *d*, $J = 6.8$ Hz, 3H-12 and 3H-13)], an olefinic methyl group [δ_H 1.78 (*dq*, $J = 2.6$ and 1.5 Hz, 3H-15)], a methine [δ_H 2.00, *septet*, $J = 6.8$ Hz, H-11)], an olefinic proton [δ_H 5.94 (*ddddq*, $J = 1.2$, 2.6, 2.8, 5.2 and 1.5 Hz, H-3)] and two hydroxyl protons [δ_H 0.99 (*s*) and 1.12 (*d*, $J = 4.1$ Hz), exchangeable with D_2O]. One of the hydroxyl groups is secondary [δ_H 3.89 (*ddd*, $J = 1.6$, 2.8 and 4.1 Hz, H-6)]. The ^{13}C NMR spectrum confirmed the presence of a trisubstituted double bond [δ_C 132.8 (*s*, C-4) and 124.3 (*d*, C-3)] and two hydroxylated carbon atoms, one tertiary [δ_C 74.9 (*s*, C-7)] and the other secondary [δ_C 75.8 (*d*, C-6)]. The molecule is therefore bicarbocyclic. A detailed decoupling study of the 360 MHz ^{1}H NMR spectrum of the diol led to structure **1** as follows.

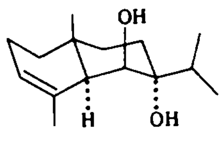
Prolonged shaking with D_2O resulted in the disappearance of the hydroxyl protons and the removal of a 4.1 Hz coupling from the secondary hydroxyl methine (H-6α). The appearance of a methine septet ($J = 6.8$ Hz) at δ2.00 coupled to the two secondary methyl groups indicated the presence of an isopropyl group attached to a fully substituted carbon atom. The tertiary methyl group resonated as a narrow triplet ($J = 0.7$ Hz) with ^4J couplings to H-1α and H-9α. The couplings of the neighbouring methylenes H-1α, H-1β, H-2α and H-2β and of H-9α, H-9β, H-8α and H-8β are readily identified. In addition to its vicinal couplings with H-2α (5.2 Hz) and H-2β (2.6 Hz) the olefinic proton H-3 exhibits long-range couplings with the olefinic methyl group (1.5 Hz), the H-5-methine (2.8 Hz) and with H-1β (1.2 Hz). In turn the olefinic



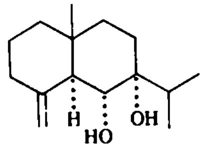
1



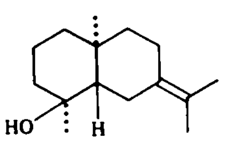
2



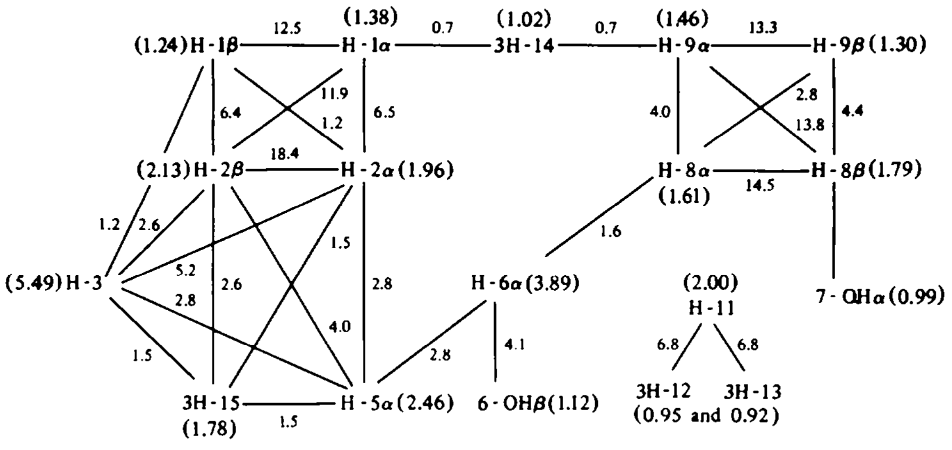
3



4



5



Scheme 1. 360 MHz ^1H chemical shifts and coupling constants of eudesm-3-ene-6β,7α-diol (1).

methyl has homoallylic couplings with H-2β (2.6 Hz) and H-2α (1.5 Hz) together with a ^4J coupling (1.5 Hz) to the H-5 methine which is also coupled homoallylically with H-2β (4.0 Hz) and H-2α (2.8 Hz) and vicinally to the secondary carbinol methine H-6α (2.8 Hz). The sequence 2 is concluded by the observation of a ^4J coupling (1.6 Hz) between H-6α and H-8α. Since a cyclopropane ring is not present the ring junction must be between C-5 and C-10 and the quaternary carbon C-7 must bear the tertiary hydroxyl and isopropyl groups. The lack of any hydrogen-bonded hydroxyl absorption in the IR spectrum requires that the hydroxyl groups are *trans*-diaxial and hence the isopropyl group is equatorial. The *trans* ring junction is required to accommodate the observed long-range couplings of H-5α and of 3H-14. These data lead unequivocally to the structure and relative configuration as in 1 for the diol. The observation of a small ^4J coupling between the tertiary hydroxyl group proton and H-8β lends further support to the assigned relative stereochemistry. The conformation which is consistent with the coupling information is shown in 3.

Attempts to establish the absolute configuration of the diol were thwarted by the lack of reactivity of the secondary hydroxyl group, which was resistant to both oxidation and acetylation, and by lack of material. The eudesmane-type sesquiterpenoids are widely distributed in the Hepaticae [3, 4] and belong to the normal [e.g. (+)-eudesmanal, (−)-frullanolide, (−)-dihydrofrullanolide, (+)-α- and (+)-β-cyclocostunolide and (+)-4-epi-arbusculin A] as well as to the enantiomeric series [e.g. (+)-α- and (−)-β-selinene, (−)-α-eudesmol (+)-frullanolide, (+)-dihydrofrullanolide and (+)-β-frullanolide] and thus the absolute configuration of the diol 1 requires independent proof. The closely related diol 4 has been isolated from the liverwort *Chiloscyphus pallescens* (Ehrh. ex Hoffm.) Dum. [5]. Again definitive evidence for its absolute configuration is lacking but in this case the authentic *ent*-eudesmenol (5) was also isolated.

A sample of *L. reptans* collected near Catrine in south-west Scotland contained beside the diol 1 an oily sesquiterpene alcohol of unknown structure.

EXPERIMENTAL

Unless otherwise stated, ^1H NMR: 360 MHz, $CDCl_3$, relative to $CHCl_3$ (δ7.25); ^{13}C NMR: 25 MHz, $CDCl_3$, TMS as int. standard.

Extraction of L. reptans. The air-dried and ground liverwort (406 g; from the Hohne-Cliffs, Harz Mountains, G.D.R.; leg. S. Huneck and J. Schmidt, 2.10.1979; det. S. H., 3.10.1979; voucher specimen deposited at the private herbarium of S.H.) was extracted with Et_2O for 24 hr. Removal of the solvent afforded an oily residue (19 g) which was chromatographed on silica gel in *n*-hexane with increasing amounts of Et_2O. *n*-Hexane (1500 ml) eluted a colourless oil (1.1 g) which consisted of β-gymnomitrene, bicyclogermacrene and cuparene according to the GC-MS analysis. *n*-Hexane–Et_2O (19:1, 1000 ml) eluted a wax (0.4 g), mp 60–65° and *n*-hexane–Et_2O (4:1, 500 ml) an oil which crystallized partly. The solid material was filtered and gave after two crystallizations from Et_2O–*n*-pentane, eudesm-3-ene-6β,7α-diol (1) (0.39 g, 0.09%) as prisms, mp 94–95°; $[\alpha]_D^{24}$ + 34.6 ($CHCl_3$; *c* 2.2), $C_{15}H_{26}O_2$ (238). IR ν_{max}^{KBr} cm^{-1}: 700, 736, 762, 794, 816, 850, 906, 936, 966, 988, 1004, 1018, 1030, 1086, 1140, 1200, 1270, 1380, 1444, 1462, 2960, 3640; EIMS *m/z* (rel. int.): 238 $[M]^+$ (4), 220 $[M-H_2O]^+$ (48), 205 $[M-H_2O-Me]^+$ (36), 177 $[M-H_2O-CHMe_2]^+$ (100), 162 (27), 159 (30), 149 (24), 147 (24), 137 (28), 135 (30), 121 (50), 119 (38), 109 (40), 107 (40); ^{13}C NMR (25 MHz, $CDCl_3$): δ15.9 (2*q*), 18.1 (*q*), 20.5 (*q*), 23.0 (*t*), 27.8 (*t*), 31.5 (*s*), 32.8 (*d*), 35.3 (*t*), 39.3 (*t*), 45.3 (*d*), 71.7 (*d*), 74.9 (*s*), 124.3 (*d*), 132.8 (*s*); ORD (MeOH): $[\alpha]_{400}^{24}$ + 22, $[\alpha]_{350}^{24}$ + 33, $[\alpha]_{300}^{24}$ + 66, $[\alpha]_{250}^{24}$ + 200, $[\alpha]_{230}^{24}$ + 555.

Further elution of the column with *n*-hexane–Et_2O (1:1, 500 ml) yielded flat needles (50 mg, from $CHCl_3$–MeOH), mp 120–122° which consisted, according to the GC-MS analysis, of stigmasterol, campesterol, sitosterol and brassicasterol [6].

A further collection of *L. reptans* (200 g) was made near Catrine in SW Scotland in Spring 1983. The usual chromatographic separation of the crude extract (6 g) yielded two major constituents: (a) eudesm-3-ene-6β,7α-diol (30 mg), identical with an authentic sample and (b) a mono-alcohol (40 mg), $C_{15}H_{26}O$, as an oil with: IR ν_{max} 3610 cm^{-1}; ^1H NMR (90 MHz, CCl_4): δ5.23 (*m*, olefinic H), 1.73 (*s* (*br*), olefinic Me), 0.89, 0.87, 0.85 (all *d*, J = 7 Hz, sec-Me); ^{13}C NMR: δ16.0 (*q*), 17.7 (2*q*), 20.6 (*q*), 23.2

(*t*), 27.4 (*t*), 35.9 (*t*), 37.7 (*d*), 37.9 (*d*), 38.7 (*t*), 45.3 (*t*), 49.2 (*s*), 85.4 (*s*), 121.5 (*d*), 139.7 (*s*).

Acknowledgements—We are grateful to Dr. J. Schmidt, Institute of Plant Biochemistry, Halle, for his help in collecting the plant material and for performing the GC-MS analysis of the sterols, to Professor Y. Asakawa (Institute of Pharmacognosy, Tokushima) for analysis of the mixture of sesquiterpenes and to Dr. I. H. Sadler and the SERC Highfield NMR Service, University of Edinburgh.

REFERENCES

1. Andersen, N. H., Bissonette, P., Liu, C. B., Shunk, B., Ohta, Y., Tseng, C. W., Moore, A. and Huneck, S. (1977) *Phytochemistry* **16**, 1731.
2. Asakawa, Y., Matsuda, R., Takemoto, T., Hattori, S., Mizutani, M., Inoue, H., Suire, C. and Huneck, S. (1981) *J. Hattori Bot. Lab.* **50**, 107.
3. Asakawa, Y. (1982) *Prog. Chem. Org. Nat. Prod.* **42**, 1.
4. Huneck, S. (1983) in *New Manual of Bryology* (Schuster, R. M., ed.) Vol. 1, p. 1. Hattori Botanical Laboratory, Nichinan, Japan.
5. Connolly, J. D., Harrison, L. J. and Rycroft, D. S. (1982) *J. Chem. Soc. Chem. Commun.* 1236.
6. Huneck, S., Janicke, S. and Schmidt, J. (1984) *Pharmazie* **39**, 784.

Phytochemistry, Vol. 25, No. 7, pp. 1747–1749, 1986.
Printed in Great Britain.

0031-9422/86 $3.00 + 0.00
Pergamon Journals Ltd.

SESQUITERPENE LACTONES FROM *LASERPITIUM GARGANICUM*

GIOVANNI APPENDINO, MARIA GRAZIA VALLE, ROSAMARIA CANIATO* and ELSA MARIA CAPPELLETTI*

Istituto Chimica Farmaceutica e Tossicologica, Facoltà di Farmacia, C.so Raffaello 31, 10125 Torino, Italy; *Dipartimento di Biologia, Università di Padova, via Orto Botanico 15, 35100 Padova, Italy

(*Received* 15 *October* 1985)

Key Word Index—*Laserpitium garganicum* subsp. *garganicum*; Umbelliferae; sesquiterpene lactones.

Abstract—Besides known compounds, the roots of *Laserpitium garganicum* subsp. *garganicum* afforded a new slovanolide triester. The locations of the ester moieties were assessed by correlation with a compound of known structure.

INTRODUCTION

The genus *Laserpitium* is a rich source of oxygenated sesquiterpenoids [1]. As a part of an investigation on plants of this genus, we have studied the constituents of *L. garganicum* (Ten.) Bertol., a species confined to the Italian peninsula and Sardinia (*L. garganicum* subsp. *garganicum*), Sicily [*L. garganicum* subsp. *siculum* (Sprengel) Pign.] and possibly the Balkans [2].

RESULTS AND DISCUSSION

Column chromatography of the chloroform extract of the roots of *L. garganicum* subsp. *garganicum* gave, along with the phenylpropane derivative laserin (**1**) [3], five sesquiterpene lactones (**2**–**6**). Lactone **2** was the eudesmanolide isosilerolide [4]. The other lactones were guaiane-type esters based on the slovanolide skeleton [5]. They differed from each other only in the nature and locations of the ester moieties, as shown by their conversion to the same rearranged triol (**7**) upon treatment with methanolic KOH [6]. Lactones **4**–**6** were identified as guaianolides previously isolated from *L. siler* L. [5]. However, lactone **3** was new. Its spectral features showed the presence of one acetyl and two angeloyl residues. The relative position of the ester moieties was established with the demonstration that acetylation of the diester **5**, a compound of known constitution [7], gave a triester (**3**) identical with the natural product.

The high concentration of sesquiterpenoids in the roots of *L. garganicum* (*ca* 5% dry wt) meant that enough of lactones **3**–**6** were isolated for ^{13}C NMR determinations. Chemical shift and multiplicity considerations as well as comparison of the spectra of triesters **3** and **4** with those of diesters **5** and **6**, allowed assignment of all signals but that of C-13, at *ca* δ20. The resonance of this carbon and those of the methyl at C-2 of the angelate(s) and the methyl of