Pergamon

PII: S0031-9422(96)00512-2

Phytochemistry, Vol. 43, No. 6, pp. 1245–1248, 1996

0031-9422/96 $15.00 + 0.00

HERBERTANE SESQUITERPENOIDS FROM THE LIVERWORTS *HERBERTUS ADUNCUS* AND *H. BOREALIS*

MALCOLM S. BUCHANAN, JOSEPH D. CONNOLLY and DAVID S. RYCROFT*

Department of Chemistry, University of Glasgow, Glasgow G12 8QQ, Scotland, U.K.

(*Received* 7 *June* 1996)

Key Word Index—*Herbertus aduncus*; *Herbertus borealis*; Herbertaceae; Jungermanniales; Hepaticae; liverwort; herbertane sesquiterpenoids; ^{1}H and ^{13}C NMR parameters.

Abstract—Two new herbertane sesquiterpenoids, (−)-1,2-dihydroxyherberten-12-al and methyl 1,2-dihydroxyherberten-12-oate, were isolated from the liverwort *Herbertus aduncus* ssp. *hutchinsiae* together with herbertene, α-herbertenol, β-herbertenol, and herbertene-1,2-diol. Their structures were established by spectroscopic methods, especially NMR spectroscopy. Herbertene and α-herbertenol were identified in *H. borealis* on analysis by GC and ^{1}H NMR. The early development of the identification of herbertane rather than cuparane sesquiterpenoids in *H. aduncus* is reviewed critically, and attention is drawn to the fact that, despite already being recognized as being obsolete, an early report concerning the occurrence of a cuparenediol in *Herbertus* species has been promulgated in a recent review.

INTRODUCTION

Among liverworts, the Herbertaceae, based on their morphology and distribution, are considered to form an ancient family in the Jungermanniales. Many members of this family have highly disjunct populations. Schuster [1] has separated *Herbertus aduncus* (Dicks.) S. F. Gray into three subspecies (while recognizing that they may not be strictly distinct) with different geographical distributions: ssp. *aduncus* from Japan and western N. America, ssp. *hutchinsiae* (Gott.) Schust. from oceanic Europe, and ssp. *tenuis* (Evans) Miller & Scott from the Appalachians.

Investigations of *H. aduncus* collected in Japan and Canada have reported compounds shown to be herbertane sesquiterpenoids either at the time of publication or subsequently [2–8]. Herbertane sesquiterpenoids have the same absolute configuration [5, 8] as *ent*-cuparanes and they provide a further significant example of the *ent*-stereospecificity of the biosynthesis of many of the liverwort sesquiterpenoids.

The literature concerning the discovery and nature of sesquiterpenoids in *Herbertus* species presents a confusing picture and a very recent review [9] has not changed this situation. Here we endeavour to clarify the position with particular reference to *H. aduncus*. The first report of the structural elucidation of a sesquiterpene with the non-isoprenoid herbertane skeleton isolated from Japanese *H. aduncus* was by Matsuo *et al.* [2] and described the hydrocarbon herbertene (**1**). Early work on *H. aduncus* (and other *Herbertus* species) relying on GC–mass spectral data misidentified the herbertane compounds as *ent*-cuparanes, including 'iso-δ-cuparenol' [3] (more conventionally cuparen-1-ol [10]). A further source of confusion is that the construction 'isocuparane' has also been applied [5, 11, 12] to the herbertane skeleton. As the prefix iso is now ambiguous in this context it would appear better to abandon the name isocuparane in favour of the name herbertane, which in any case has priority in terms of publication date [2]. Most of the *ent*-cuparanes claimed have been revised to herbertanes, although the only explicit statement published about the revisions refers to only one cuparene derivative in a comment on mass spectral similarities [5]. The revisions can be deduced to be as follows: an 'unidentified' sesquiterpenoid ($[M]^+$, $m/z = 218$) was first given the name herbertusone [4], then assigned [3] as δ-cuparenol (more conventionally cuparen-2-ol [10]) and described as a major component [13], and finally tacitly identified in print (by virtue of being shown as a major component and identification of the plant collection site and date) as α-herbertenol (**2**) (but given the name 4-hydroxyisocuparene) [5] after structure **2** had been published [6]; similarly the minor alcohol previously assigned tentatively [3] as iso-δ-cuparenol was identified tacitly [5] as β-herbertenol (**3**). At the same time as α-herbertenol (**2**) was recognized a compound present in similar abundance (and presumably corresponding to the compound in the same extract labelled 'herbertusolide' in the original paper [4]) was assigned [5] as 2,3-dihydroxycuparene (more conventionally cuparene-1,2-diol [10]); this was before the structure of herbertene-1,2-diol (**4**) had been published [7], and

*Author to whom correspondence should be addressed.

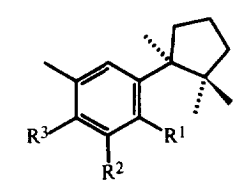
subsequently [12, 14] structure **4** was substituted tacitly for the cuparenediol structure (deducible because ref. 5 does not mention herbertene-1,2,-diol **4**, yet Nagashima *et al.* [12] and Asakawa *et al.* [14] both cite Asakawa *et al.* [5] as a source of data for compound **4**). In view of these revisions, it is erroneous for the recent review [9] by one of the authors of refs [12] and [14] to cite ref. [5] in support of statements that '2,3-dihydroxycuparene' is produced by *H. aduncus*. There is no substantiated, published evidence remaining for the occurrence of any cuparenes in *H. aduncus*.

H. aduncus ssp. *hutchinsiae*, *H. borealis* Crundw. and *H. stramineus* (Dum.) Lett are the species of the Herbertaceae which grow in Scotland [15], and no chemical investigations have been reported. *H. borealis* is an extremely rare species of liverwort and its only known British location is in a nature reserve on Beinn Eighe in north-west Scotland (this site contains virtually the entire world population as it is known otherwise only from three small sites in south-west Norway). Within the Beinn Eighe plant community it is conspicuous and can be locally dominant. *H. borealis* was described as a new species in 1970 by Crundwell [16], having previously been referred to other *Herbertus* species. In this paper, we report the results of a study of *H. aduncus* ssp. *hutchinsiae* and *H. borealis*.

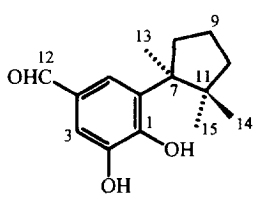
RESULTS AND DISCUSSION

From an ether extract of Scottish *H. aduncus*, six herbertane sesquiterpenoids (**1–6**) were isolated, two of which (**5**, **6**) are new. The other four, (−)-herbertene (**1**), (−)-α-herbertenol (**2**), (−)-β-herbertenol (**3**) and (−)-herbertenediol (**4**), were readily identified by comparison of their physical and spectroscopic data with published data [8].

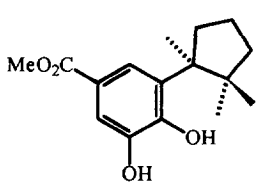
Compound **5** is a crystalline compound and from HR-MS has the molecular formula $C_{15}H_{20}O_3$. Its IR spectrum revealed the presence of hydroxyl and carbonyl groups. The ^{1}H NMR spectrum has resonances for three tertiary methyls, two aromatic protons which are *meta*-related, and an aldehyde proton. The ^{13}C NMR spectrum has resonances for 15 carbons and confirmed the presence of an aldehyde and a tetrasubstituted benzene. It contains further signals for three methyls, three methylenes, and two quaternary sp^3 carbons. These data reveal that this compound is an aromatic bicyclic sesquiterpenoid containing a 1,2,3,5-tetrasubstituted benzene nucleus with an aldehyde group, two hydroxyls and a cyclopentane ring bearing three tertiary methyls. These data indicate a cuparane or herbertane skeleton.



	R^1	R^2	R^3
1	H	H	H
2	OH	H	H
3	H	H	OH
4	OH	OH	H



5



6

Signal assignments and the position of attachment of the aromatic substituents were established as follows. Molecular models indicate that the most shielded methyl group (δ_H 0.74) is H_3-13 since it lies closest to the shielding zone of the benzene ring. The NOEs observed between H_3-13 and the signal at δ_H 1.19 (*s*, H_3) indicate that the latter is H_3-15 and therefore the remaining tertiary methyl signal at δ_H 1.44 must be H_3-14. This signal is notably deshielded since the H_3-14 methyl group lies in the deshielding zone of the aromatic ring. A similar situation pertains for H-8α which resonates at δ_H 2.66 (*m*) – more deshielded than any of the other methylene protons. The α-configuration of this proton is confirmed by NOEs observed between H_3-13 and H-8α. One of the aromatic protons (δ_H 7.40) received NOEs from H_3-14, H_3-13, H-8α and the aldehyde proton indicating that it must be *ortho* to both the cyclopentane ring and the aldehyde proton. Because the aromatic protons are *meta*-coupled the above information leads to either a 1,2- or a 2,3-dihydroxyherbertene derivative. Both aromatic protons are deshielded compared to those in herbertene-1,2-diol (**4**) [8] and herbertene-2,3-diol [11], and both are deduced to be *ortho* to the aldehyde. Thus compound **5** is (−)-1,2-dihydroxyherberten-12-al. The specific rotation $[\alpha]_D$ of compound **5** is −11°; herbertanes generally have small negative rotations [8] and it is assumed that compound **5** belongs to the same absolute stereochemical series.

Compound **6** has the molecular formula $C_{16}H_{22}O_4$ (HR-mass spectrometry). Its NMR data are very similar to those of compound **1**, except for the presence of a carbomethoxyl group in place of the aldehyde group. This information indicates that compound **6** is methyl 1,2-dihydroxyherberten-12-oate.

Fractions containing fatty acid esters were also isolated from the extract. One fraction contained a mixture of fatty acid esters of a triterpenoid, readily identified as cycloartenol by comparison of its spectroscopic properties with literature values [17]. Cycloartenol itself was isolated from a later fraction. Methanolysis of the mixture followed by GC and GC–mass spectral analysis with appropriate standards resulted in the identification of the methyl esters of linoleic, α-linolenic, arachidonic, eicosa-5*Z*,8*Z*,11*Z*,14*Z*,17*Z*-pentaenoic, palmitic and isostearic acids. The triterpenoid obtained from the methanolysis experiment was largely 25-methoxycycloartanol, an artefact of the reaction conditions.

The other fatty acid ester fraction contained triglycerides. Six olefinic doublet resonances in the ^{13}C NMR spectrum showed that the predominant fatty acid

moiety was α-linolenic acid [18]. GC analysis of the fatty acid methyl esters obtained by methanolysis confirmed this and also revealed the presence of the five other acid moieties already identified in the cycloartenol ester fraction.

The ether extract of the second species investigated, *H. borealis*, was subjected to vacuum distillation to separate the more volatile terpenoid fraction. The composition of this terpenoid fraction was investigated using GC and comparison with authentic specimens. The mixture was found to consist largely of herbertene (**1**) and α-herbertenol (**2**). This was confirmed from the ^{1}H NMR spectrum of the terpenoid mixture which contained readily identifiable signals for both compound **1** and compound **2** [8].

In conclusion, in common with other *Herbertus* species [9], the Scottish collections of *H. aduncus* and *H. borealis* contain herbertane sesquiterpenoids. A new herbertanoid aldehyde (**5**) is the major sesquiterpenoid in the case of *H. aduncus*, but not *H. borealis*. The discovery of compound **5** in Scottish *H. aduncus* is notable in connection with Schuster's recognition of *H. aduncus* from oceanic Europe as subspecies *hutchinsiae* [1], and it would be worthwhile to examine *H. aduncus* from other parts of Britain and Europe to find out if the presence of compound **5** is a constant character. We have found aldehyde **5** to be present in extracts of the three other collections of *H. aduncus* from the West of Scotland that we have investigated [19]: it was also present in fresh *H. aduncus* from Glen Etive, Argyll, and in a herbarium sample of *H. aduncus* from Beinn Eighe, but was not observed in herbarium samples of *H. borealis* from Beinn Eighe (D.S.R. unpublished data). No ent-cuparanes were detected.

EXPERIMENTAL

General. TLC: over Merck precoated silica gel 60 F_{254}, visualized under UV light (254 nm) and by spraying with 25% H_2SO_4 and heating. Flash CC and prep. TLC: silica gel GF_{254}. Mps uncorr. GC: CP Sil 5 CB (chrompack) fused silica gel capillary column (25 m × 0.32 mm i.d. × 0.12 μm) and FID. The Grob-type injector was operated in the split mode (50:1) and the He carrier and make up gas flow rate was 2 ml min^{-1}. A linear temp. programme was used in which the column temp. was programmed from 80° (2 mins) to 240° (5 mins) at 5° min^{-1}. The injection port and detector temp. were 255° and 260°, respectively. NMR spectra (^{1}H, 200 MHz; ^{13}C, 50 MHz) were recorded for $CDCl_3$ solutions relative to $CHCl_3$ at δ_H 7.25 and $CDCl_3$ at δ_C 77.0. Assignment of ^{1}H NMR signals was aided by NOE difference experiments. Multiplicities were determined from DEPT experiments. IR spectra were measured for CCl_4, UV for MeOH and $[\alpha]_D$ for $CHCl_3$ solutions. EI-MS at 70 eV.

Plant material. Herbertus aduncus was collected by D.S.R. at Loch Creran, Argyll, Scotland, on 29th September 1991; *H. borealis* was collected by D.S.R. at Beinn Eighe National Nature Reserve, Wester Ross, Scotland, on 7th April 1992. Voucher specimens are deposited in the herbarium of the Division of Environmental and Evolutionary Biology, Institute of Biomedical and Life Sciences, University of Glasgow.

Extraction of H. aduncus. The material was ground mechanically and the powder (660 g) extracted with Et_2O to yield 15.5 g of crude extract which was fractionated by CC over silica gel using a petrol–Et_2O gradient. The fractions were rechromatographed by prep. TLC over silica gel (petrol–Et_2O, CH_2Cl_2–Et_2O, *n*-hexane-CH_2Cl_2) to give the following constituents in order of increasing polarity: a mixt. of alkanes and terpene hydrocarbons (700 mg), (−)-herbertene (**1**) (29 mg), a cycloartenol-fatty acid ester mixture (2.10 g) [17], (−)-α-herbertenol (**2**) (1.10 g), triglycerides (1.25 g), (−)-β-herbertenol (**3**) (152 mg), (−)-herbertenediol (**4**) (410 mg), β-sitostenone (4 mg) [20] cycloartenol (74 mg) [17], methyl 1,2-dihydroxyherberten-12-oate (**6**) (11 mg) and (−)-1,2-dihydroxyherberten-12-al (**5**) (1.32 g).

(−)-*1,2-Dihydroxyherberten-12-al* (**5**). Crystals from CH_2Cl_2, mp 153–155°. $[\alpha]_D$ − 11° ($CHCl_3$, *c*, 1.5). HRMS *m/z*: 248.1407 $[M]^+$ ($C_{15}H_{20}O_3$ requires 248.1421); EIMS *m/z* (rel. int.): 248 $[M]^+$ (78), 178 (100), 166 (75), 165 (81), 137 (16), 92 (16), 77 (14); UV λ_{max} nm: 232, 292, 310; IR ν_{max} cm^{-1}: 3670, 3590, 3500, 3350, 3200 (OH); 1670 (C=O); ^{1}H NMR: δ_H 9.69 (*s*, H-12), 7.40 (*d*, *J* = 1.9 Hz, H-5), 7.24 (*d*, *J* = 1.9 Hz, H-3), 2.66 (*m*, H-8α), 1.45–1.90 (*m*, 5H), 1.44 (*s*, 3H-14), 1.19 (*s*, 3H-15), 0.74 (*s*, 3H-13); ^{13}C NMR (3 drops of CD_3OD were added to the $CDCl_3$ solution): δ_C 193.0 (*d*), 151.6 (*s*), 144.4 (*s*), 133.4 (*s*), 127.9 (*d*), 127.4 (*s*), 110.7 (*d*), 51.2 (*s*), 44.8 (*s*), 41.1 (*t*), 39.1 (*t*), 27.0 (*q*), 25.5 (*q*), 22.2 (*q*), 20.3 (*t*).

Methyl 1,2-dihydroxyherberten-12-oate (**6**). Gum. HRMS *m/z*: 278.1512 $[M]^+$ ($C_{16}H_{22}O_4$ requires 278.1518). EIMS *m/z* (rel. int.): 278 $[M]^+$ (62), 208 (96), 196 (79), 195 (100), 177 (41), 137 (30), 91 (21), 77 (17); UV λ_{max} nm: 228, 268, 288. IR ν_{max} cm^{-1}: 3680, 3600, 3510, 3360, 3200 (OH); 1690 (C=O); ^{1}H NMR: δ_H 7.66 (*d*, *J* = 1.9 Hz, H-5), 7.49 (*d*, *J* = 1.9 Hz, H-3), 3.87 (*s*, OMe), 2.62 (*m*, H-8α), 1.45-1.90 (*m*, 5H), 1.41 (*s*, 3H-14), 1.18 (3H-15); 0.72 (*s*, 3H-13); ^{13}C NMR: δ_C 167.9 (*s*), 148.8 (*s*), 142.9 (*s*), 133.2 (*s*), 124.0 (*d*), 119.9 (*s*), 113.7 (*d*), 52.1 (*q*), 51.3 (*s*), 44.8 (*s*), 41.1 (*t*), 39.2 (*t*), 27.0 (*q*), 25.5 (*q*), 22.4 (*q*), 20.3 (*t*).

Methanolysis of the triterpenoid esters. The ester mixture (20 mg) was refluxed with acetyl chloride (1 ml), MeOH (25 ml) and Et_2O (3 ml) for 5 hr. The solvents were evapd and the products separated by prep. TLC (Et_2O–petrol, 1:4) to give a fatty acid methyl ester mixture and a triterpenoid alcohol mixture. The esters were identified by comparison of R_t (GC) and mass spectral fragmentation patterns (GC-MS) with those of authentic specimens and were methyl esters of the following acids: R_t mins (rel. abund.): palmitic 11.64 (9%), linoleic 15.45 (21%), α-linolenic 15.55 (33%), isostearic 16.57 (5%), arachidonic 20.00 (15%), and eicosa-5Z,8Z,11Z,14Z,17Z-pentaenoic 20.80

(7%), unidentified 10% including 14.87 (3%). The triterpenoid alcohol mixture contained largely 25-methoxycycloartanol, identified by comparison with cycloartenol [17]: δ_H 3.17 (*s*, OMe), 1.13 (*s*, Me-26 and Me-27); δ_C 74.7 (*s*, C-25), 49.1 (*q*, OMe), 25.0 (*q*, C-26 and C-27).

Methanolysis of the triglyceride esters. The same procedure as for the triterpenoid esters was used. Analysis by GC and comparison of R_t values showed that esters of the six acid moieties found in the cycloartenol ester mixture constituted 86% of the methyl esters, namely, the methyl esters of palmitic (7%), linoleic (11%), α-linolenic (61%), isostearic (< 1%), arachidonic (5%), and eicosa-5Z,8Z,11Z, 14Z,17Z-pentaenoic (3%) acids; the remainder, principally R_t 11.11 (3%) and 15.84 min (4%), were unidentified.

Extraction of H. borealis. The dried plant material (120 g) was ground and extracted with Et_2O to yield 690 mg of crude extract. The extract (*ca* 160 mg) was subjected to vacuum distillation and gave a very small quantity (<1 mg) of a terpene mixture, which was analysed by means of GC (comparison with authentic specimens) and ^{1}H NMR [8]. From the data obtained two main terpenoid components were identified as herbertene (**3**) (R_t 12.39 min) and α-herbertenol (**4**) (R_t 17.83 min). From GC peak areas the ratio of compound **3** to compound **4** was 3:4.

Acknowledgements—We thank the Loudon Trust and the Buchanan Society for a studentship and a bursary respectively (to M.S.B.), Dr W. J. Cole for GC, and Scottish Natural Heritage for permitting (and Mr Tim Clifford, Anancaun Field Station, for supervising) the collection of *Herbertus borealis*.

REFERENCES

1. Schuster, R. M. (1966) *The Hepaticae and Anthocerotae of North America East of the Hundredth Meridian*, Vol. I, p. 712. Columbia University Press, New York.
2. Matsuo, A., Yuki, S., Nakayama, M. and Hayashi, S. (1981) *J. Chem. Soc. Chem. Comm.* 864.
3. Asakawa, Y., Matsuda, R., Toyota, M., Suire, C., Takemoto, T., Inoue, H., Hattori, S. and Mizutani, M. (1981) *J. Hattori Bot. Lab.* **50**, 165.
4. Asakawa, Y., Tokunaga, N., Toyota, M., Takemoto, T., Hattori, S., Mizutani, M. and Suire, C. (1979) *J. Hattori Bot. Lab.* **46**, 67.
5. Asakawa, Y., Matsuda, R., Schofield, W. B. and Gradstein, S. R. (1982) *Phytochemistry* **21**, 2471.
6. Matsuo, A., Yuki, S., Nakayama, M. and Hayashi, S. (1982) *Chem. Letters* 463.
7. Matsuo, A., Yuki, S. and Nakayama, M. (1983) *Chem. Letters* 1041.
8. Matsuo, A., Yuki, S. and Nakayama, M. (1986) *J. Chem. Soc. Perkin Trans. 1* 710.
9. Asakawa, Y. (1995) *Progress in the Chemistry of Organic Natural Products* (Herz, W., Kirby, G. W., Moore, R. E., Steglich, W. and Tamm, Ch., eds) Vol. 65, p. 1. Springer, Wien.
10. Buckingham, J. (ed.) (1994) *Dictionary of Natural Products*, Vol. 7, p. lxviii. Chapman & Hall, London.
11. Fukuyama, Y. and Asakawa, Y (1991) *J. Chem. Soc. Perkin Trans. 1* 2737.
12. Nagashima, F., Nishioka, E., Kameo, K., Nakagawa, C. and Asakawa, Y. (1991) *Phytochemistry* **30**, 215.
13. Gradstein, S. R., Matsuda, R. and Asakawa, Y. (1981) *J. Hattori Bot. Lab.* **50**, 231.
14. Asakawa, Y., Lin, X., Kondo, K. and Fukuyama, Y. (1991) *Phytochemistry* **30**, 4019.
15. Smith, A. J. E. (1990) *The Liverworts of Britain and Ireland.* Cambridge University Press, Cambridge.
16. Crundwell, A. C. (1970) *Trans. Br. Bryol. Soc.* **6**, 41.
17. Seo, S., Uomori, A., Yoshimura, Y., Takeda, K., Seto, H., Ebizuka, Y., Noguchi, H. and Sankawa, U. (1988) *J. Chem. Soc. Perkin Trans. 1* 2407.
18. Johns, S. R., Leslie, D. R., Willing, R. I. and Bishop, D. G. (1977) *Aust. J. Chem.* **30**, 813.
19. Buchanan, M. S. (1994) Ph.D. thesis. University of Glasgow, Scotland.
20. Joshi, K. C., Bansal, R. K. and Singh, P. (1974) *Indian J. Chem.* **12**, 903.