

## Chemical Constituents of *Herbertus dicramus*, Three New Herbertene Type Sesquiterpenes

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Three new herbertane-type sesquiterpenes, 2-hydroxyherbertene, methyl-2-hydroxyherberten-12-oate and methyl-2-methoxyherberten-12-oate have been isolated from the extract of *Herbertus dicramus*. Two known compounds, (-)- $\alpha$ -herbertenol and (-)- $\beta$ -herbertenol, were also present in the extract of *Herbertus dicramus*. The structures of the new compounds were elucidated with the help of  $^1\text{H}$ ,  $^{13}\text{C}$  NMR, two dimensional NMR and mass spectroscopy.

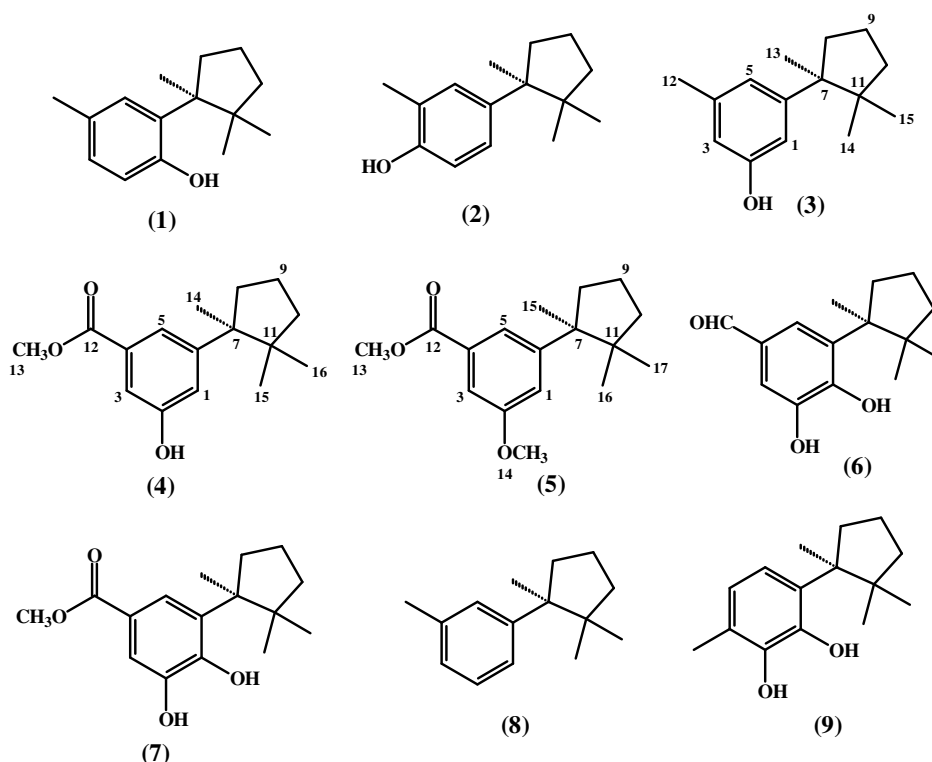
**Key Words:** *Herbertus dicramus*, Herbertene, Sesquiterpene, Isolation, Structure.

### INTRODUCTION

Natural products extracted from plants, animals and lesser organisms attracted great interest by the scientists since they were used in treatments for diseases as poisons, as euphoriant and as stimulants. This interest has increased over the years with the development of separation and purification methods, such as column chromatography, GC, TLC, HPLC paper chromatography, ion exchange, etc. Following the separation and purification of compounds, different spectroscopic techniques are used for structure elucidation, such as NMR (including two dimensional NMR spectroscopy; COSY, HMBC, HSQC), IR, UV and mass spectroscopy. Among liverworts, the herbertaceae is considered to form an ancient family in the *Jungermanniales*, based on their morphology and distribution<sup>1</sup>. Liverworts from the genus *Herbertus* contain herbertane-type sesquiterpenoids that can be considered as chemical markers of the genus<sup>2,3</sup>.

In this paper, the chemical constituents of *Herbertus dicramus*, collected in Scotland, are revealed for the first time. The extract gave (-)- $\alpha$ -herbertenol (**1**), (-)- $\beta$ -herbertenol (**2**) and the new herbertane-type sesquiterpenes, 2-hydroxyherbertene (**3**), methyl-2-hydroxyherberten-12-oate (**4**) and methyl-2-methoxyherberten-12-oate (**5**) as colourless oils. Previous investigation of Scottish *H. Aduncus* by Buchanan *et al.*<sup>1</sup> had led to the isolation of two herbertane sesquiterpenoids (-)-1,2-dihydroxyherberten-12-al (**6**) and methyl-1,2-dihydroxyherberten-12-oate (**7**), which have similarities with the structures of compounds (**4**) and (**5**). Among the other related species known in the genus *Herbertus*, Matsuo *et al.*<sup>4</sup> have examined the liverwort *Herbertus aduncus* and isolated a sesquiterpene which they called (-)-herbertene (**8**). Asakawa

*et al.*<sup>5</sup> examined *H. aduncus*, *H. sakuraii* and *H. subdentatus* collected in Canada, Colombia and Japan. These three species were chemically quite similar to each other. They all synthesized (-)- $\alpha$ -herbertenol (**1**), (-)- $\beta$ -herbertenol (**2**) and herbertene (**8**). *H. aduncus* and *H. subdentatus* also synthesized (-)-herbertenediol (**9**), which was named as 2,3-dihydroxycuparene at the time by Asakawa *et al.*<sup>5</sup> and they have concluded that herbertane- and cuparane-type sesquiterpenoids are characteristic constituents of *Herbertus* species<sup>5</sup>. However there is no published evidence from the occurrence of any cuparenes in *Herbertus* species<sup>1</sup>.



## EXPERIMENTAL

The crude extract was fractionated by column chromatography over silica gel G<sub>254</sub> and the further purification was done on TLC by using 0.75 mm thick preparative plates. The eluents used were increasing percentages of ethyl acetate in light petroleum. Compounds on TLC and analytical plates were visualized using UV light or iodine vapour. The solvents were either of analytical grade or bulk solvents distilled before use. Nuclear magnetic resonance spectra (NMR) were recorded on Burker WP AM 360 (<sup>1</sup>H at 360 MHz and <sup>13</sup>C at 90 MHz). Spectra were recorded for CHCl<sub>3</sub> relative to  $\delta_{\text{H}}$  7.25 and CDCl<sub>3</sub> relative to  $\delta_{\text{C}}$  77.0 and chemical shifts are reported in ppm. <sup>1</sup>H and <sup>13</sup>C signal assignments are based on general chemical shift rules and comparison with published data for similar compounds. Infrared spectra were recorded in CHCl<sub>3</sub>

solution on Perkin-Elmer 580 spectrometer and ultra-violet spectra were measured in ethanol solutions using Perkin-Elmer Lambda 9 UV-vis/NIR spectrometer. Mass spectra were determined on a Kratos MS9 instrument.

## RESULTS AND DISCUSSION

The plant *Herbertus dicramus* was collected in Scotland and identified at the University of Glasgow. The plant material was dried, ground and extracted with diethyl ether. The total crude extract gained was 2.5 g from 150 g plant material. Crude extract was first examined by analytical TLC, which showed several spots under the UV light, then subjected to flash chromatography with increasing percentages of ethyl acetate in petroleum ether. Further purification was carried out by preparative TLC. Two main constituents, (-)- $\alpha$ -herbertenol (**1**) and (-)- $\beta$ -herbertenol (**2**) were present in the extract eluted with 20 and 25 % ethyl acetate and petroleum ether. Their structures were determined by comparison of their NMR data with those reported in the literature<sup>6,7</sup>. The mass spectra of the samples supported the molecular formula of  $\alpha$ - and  $\beta$ -herbertenols, C<sub>15</sub>H<sub>22</sub>O, by showing parent ion peaks at 218 m/z. The UV spectra of the samples in ethanol had bands at 210 and 230 nm. The NMR data of  $\alpha$ - and  $\beta$ -herbertenols found in this plant are given in Table-1.

TABLE-1  
<sup>1</sup>H AND <sup>13</sup>C NMR DATA OF (-)- $\alpha$ -HERBERTENOL (**1**) AND (-)- $\beta$ -HERBERTENOL (**2**)

(-)- $\alpha$ -Herbertenol ( <b>1</b> )		(-)- $\beta$ -herbertenol ( <b>2</b> )		
$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$	
–	152.3	1	126.9	7.04 (dd, $J = 8.4$ Hz, 2.4 Hz)
6.56 (d, $J = 8.0$ Hz)	116.7	2	116.0	6.67 (d, $J = 8.3$ Hz)
6.84 (dd, $J = 8.0$ Hz, 2.0 Hz)	127.2	3	151.7	–
–	128.9	4	129.4	–
7.09 (d, $J = 1.7$ Hz)	130.0	5	129.7	7.07 (s)
–	133.0	6	132.8	–
–	50.9	7	51.0	–
1.73 (m)	20.3	8	19.9	1.71 (m)
1.77 (m)	39.4	9	40.0	1.75 (m)
1.55 (m)	41.2	10	40.9	1.56 (m)
–	44.7	11	43.5	–
2.25 (s)	20.9	12	21.4	2.23 (s)
1.40 (s)	22.9	13	23.4	0.55 (s)
1.18 (s)	25.5	14	26.0	1.22 (s)
0.75 (s)	27.0	15	26.7	1.03 (s)
4.50 (brs)	–	OH	–	4.53 (brs)

The new sesquiterpene, 2-hydroxyherbertene (**3**) was present in the fraction eluted with 30 % ethyl acetate and petroleum ether and it had a similar <sup>1</sup>H NMR spectrum to those of  $\alpha$ - and  $\beta$ -herbertenols. The main difference concerned the proton signals of the benzene ring. The signals of the three benzene protons were

now seen as broad singlets, at  $\delta_H$  6.73, 6.63 and at 6.47, which showed that the hydroxyl must be attached to C-2 on the ring, leaving a 1,3,5-trisubstituted benzene ring. The rest of the spectrum showed the four methyls, one of which is attached to the benzene ring, at  $\delta_H$  2.28, 1.36, 1.20 and at 0.71. The hydroxyl proton appeared at 4.53 as broad singlet. Its mass spectrum showed a parent ion peak,  $[M^+]$ , at 218 m/z supporting the molecular formula,  $C_{15}H_{22}O$ , for this new compound, named as 2-hydroxyherbertene (**3**). Its UV spectrum, in ethanol, showed bands at 210 and at 230 nm. The HMBC spectrum showed clear correlations supporting the 1,3,5-trisubstituted benzene ring present in the molecular structure; H-1 had correlations with C-6 and C-2, H-3 with C-2, C-4 and C-12 and H-5 had correlations with C-4, C-6 and C-12. The rest of the correlations and the results of  $^{13}C$  and  $^1H$  NMR spectra of this new compound are given in the Table-2.

TABLE-2  
NMR DATA OF 2-HYDROXYHERBERTENE (**3**)

	$\delta_C$	$\delta_C$	HMBC correlations
1	116.9	6.73 (brs)	
2	153.0		H-1, H-3
3	117.0	6.63 (brs)	3H-12
4	128.0		H-3, H-5, 3H-12
5	130.2	6.47 (brs)	3H-12
6	13.8		H-1, H-5, 3H-13
7	50.7		H-1, 2H-8, 3H-13, 3H-14, 3H-15
8	20.2	1.72 (m)	2H-9, 3H-13
9	38.8	1.77 (m)	2H-8, 2H-10
10	41.0	1.56 (m)	2H-9, 3H-14, 3H-15
11	43.9		2H-8, 2H-9, 2H-10, 3H-14, 3H-15
12	20.2	2.28 (s)	H-3, H-5
13	21.8	1.36 (s)	2H-8
14	25.0	1.20 (s)	2H-10, 3H-15
15	27.3	0.71 (s)	2H-10, 3H-14

The other two herbertane-type sesquiterpenes methyl-2-hydroxyherberten-12-oate (**4**) and methyl-2-methoxyherberten-12-oate (**5**) were present in the fractions eluted with 45 and 50 % ethyl acetate and petroleum ether. Most of the spectral data of methyl-2-hydroxyherberten-12-oate was similar to those of 2-hydroxyherbertene, however, its  $^{13}C$  NMR spectrum showed 16 carbons, one of which is an ester carbonyl carbon giving signal at  $\delta_C$  168.5 and another signal observed at  $\delta_C$  51.3 belonged to the methyl carbon in the ester group. Aromatic carbons were seen between  $\delta_C$  117.9 and  $\delta_C$  155.1 and the other methyl carbons, C-14, C-15 and C-16 were seen, respectively at  $\delta_C$  22,  $\delta_C$  24.7 and at  $\delta_C$  27.5. Its  $^1H$  NMR spectrum, on the other hand, showed a singlet at  $\delta_H$  4.30 belonging to the methyl protons of the

ester group and the broad singlet at  $\delta_{\text{H}}$  4.91 was the hydroxyl group present in the structure. The mass spectrum also supported the molecular formula,  $\text{C}_{16}\text{H}_{22}\text{O}_3$ , by showing a parent ion peak at 262.1 m/z. The UV spectrum had bands at 220 and 230 nm. Its IR spectrum showed bands at 3010, 1710, 1590, 1410 and 1390  $\text{cm}^{-1}$ . The  $^1\text{H}$  NMR spectrum of methyl-2-methoxyherberten-12-oate (**5**),  $[\text{M}^+]$  276 m/z, showed no signal of a hydroxyl group, but the rest of the proton spectrum was almost identical with those of methyl-2-hydroxyherberten-12-oate. The only difference was the sharp singlet seen at  $\delta_{\text{H}}$  3.88, which belonged to the methoxy group. Its  $^{13}\text{C}$  NMR spectrum showed the ester group at  $\delta_{\text{C}}$  168.2 and at  $\delta_{\text{C}}$  51.2, aromatic carbons were seen between  $\delta_{\text{C}}$  116.8 and at  $\delta_{\text{C}}$  155.1 and the signal seen at  $\delta_{\text{C}}$  57.4 belonged to the methoxy carbon attached to the aromatic ring at C-2, leaving a trisubstituted benzene ring, which was also observed in its  $^1\text{H}$  NMR spectrum. The bands 217 and 235 nm were seen in the UV spectrum and the IR showed bands at 3015, 1720, 1605, 1390, 1370  $\text{cm}^{-1}$ . Heteronuclear multiple bond correlation experiments of compound (**5**) showed weak correlations of the methoxy protons with C-1 and C-3 in the aromatic ring and the rest of the spectral data are given in Table-3.

TABLE-3  
NMR DATA OF METHYL-2-HYDROXYHERBERTEN-12-OATE (**4**)  
AND METHYL-2-METHOXYHERBERTEN-12-OATE (**5**)

Methyl-2-hydroxyherberten-12-oate	Methyl-2-methoxyherberten-12-oate					
HMBC correlations	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$	HMBC correlations	
H-3, H-5	7.0 (brs)	117.9	1	116.8	7.0 (brs)	3H-14, H-3, H-5
H-1, H-3		155.1	2	155.1		H-1, H-3
	7.40 (brs)	130.0	3	129.8	7.34 (brs)	3H-14
3H-13, H-3, H-5		130.9	4	130.7		3H-13, H-3, H-5
	7.61 (brs)	128.4	5	129.0	7.80 (brs)	
H-1, H-5, 3H-14		132.1	6	132.0		H-1, H-5, 3H-15
H-1, 2H-8, 3H-14, 3H-15		49.0	7	49.1		H-1, 2H-8, 3H-15
2H-9, 3H-14	1.70 (m)	20.5	8	20.8	1.68 (m)	2H-9, 3H-15
2H-8, 2H-10	1.75 (m)	38.6	9	38.9	1.74 (m)	2H-8, 2H-10
2H-9, 3H-15, 3H-16	1.57 (m)	40.0	10	40.2	1.56 (m)	2H-9, 3H-16, 3H-17
2H-9, 2H-10, 3H-15, 3H-16		43.8	11	44.0		2H-9, 2H-10, 3H-16, 3H-17
3H-13, H-3, H-5		168.5	12	168.2		3H-13, H-3, H-5
	4.30 (s)	51.3	13	51.2	4.32 (s)	
2H-8	1.38 (s)	22.0	14	57.4	3.88 (s)	H-1, H-3
2H-10, 3H-16	1.21 (s)	24.7	15	22.2	1.37 (s)	2H-8
2H-10, 3H-15	0.85 (s)	27.5	16	24.4	1.18 (s)	2H-10, 3H-17
			17	27.5	0.92 (s)	2H-10, 3H-16

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