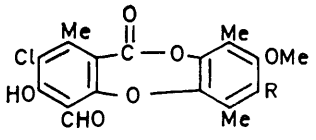
Structures of the Lichen Depsidones Granulatin and Chlorogranulatin

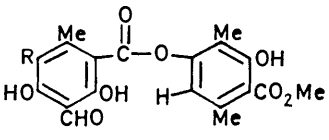
By **Eng Meng Goh** and **Alistair L. Wilkins,*** Chemistry Department, University of Waikato, Hamilton, New Zealand

The lichen depsidones granulatin and chlorogranulatin, isolated from *Pseudocyphellaria granulata* and *P. faveolata*, are identified as methyl 4-formyl-3,8-dihydroxy-1,6,9-trimethyl-11-oxo-11*H*-dibenzo[*b*,*e*][1,4]dioxepin-7-carboxylate (5a), and methyl 2-chloro-4-formyl-3,8-dihydroxy-1,6,9-trimethyl-11-oxo-11*H*-dibenzo[*b*,*e*][1,4]-dioxepin-7-carboxylate (5b), respectively. The latter is a new depsidone metabolite which has hitherto been confused with pannarin (1a).

In the course of a chemotaxonomic survey of some South American lichens of the genus *Pseudocyphellaria*, Huneck *et al.*[1] noted the occurrence in a specimen determined as *P. physciopora* Nyl. of a substance presumed to be the depsidone pannarin (1a).[2,3] In an earlier investigation of some New Zealand species of *Pseudocyphellaria*, Murray [4] isolated from a specimen that he determined as *P. flotowiana* (Laur.) Malme, but now referred to as *P. faveolata* (Del.) Malme, a chlorinated substance which appeared to analyse for $C_{19}H_{17}ClO_8$. Because the melting point of Murray's substance was close to that of the depside chloroatranorin (2b) [5] and also because of a similar correspondence in the melting point of chloroatranol [6] with the substance prepared in a like manner [6] from Murray's substance, the chlorinated metabolite present in the New Zealand lichen was considered to be chloroatranorin. More recently however it has emerged that whilst two phenolic aldehydes are present in some New Zealand and South American *Pseudocyphellaria* species, neither of these aldehydic substances is identical with chloroatranorin (2b) or pannarin (1a). In order



(1)
a; R = H
b; R = Cl



(2)
a; R = H
b; R = Cl

that the identities of the two lichen substances might be resolved, the extractions of *P. granulata* (Bab. in Hook. f.) Malme and *P. faveolata* have been undertaken.

Soxhlet extraction of the finely ground lichen materials with light petroleum gave extractives which after chromatographic separation yielded two phenolic aldehydes, $C_{19}H_{16}O_8$, m.p. 214—215°, and $C_{19}H_{15}ClO_8$, m.p. 202—203°, designated granulatin and chlorogranulatin, respectively. Also isolated was a triterpenoid triol, $C_{30}H_{52}O_3$, m.p. 226—228°, which proved to be identical with a specimen of hopane-6α,7β,22-triol isolated previously [7] from *P. crocata* (L.) Wain. (syn. *P. mougeotiana* var. dissecta Del). Subsequent extraction of the lichen material with acetone yielded further quantities of hopane-6α,7β,22-triol, and the depsidones, stictic acid, norstictic acid, and constictic acid.

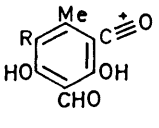
That granulatin and chlorogranulatin were also depsidones followed from the presence in their i.r. spectra of intense absorptions in the region $\nu_{max.}$ (KBr) 1 740—1 745 cm^{-1} (depsidone CO). In each of the compounds there also occurred absorptions in the regions $\nu_{max.}$ 3 570—3 200, 1 668—1 642, and 1 260—1 252 cm^{-1}. These data suggested the presence of *o*-hydroxy-aldehyde and/or *o*-hydroxy-ester groupings in each of the compounds. ^1H N.m.r. data (Table) confirmed the presence

^1H N.m.r. assignments [δ (p.p.m.) in $CDCl_3$]

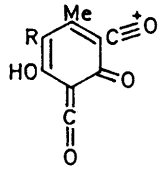
	Compound			
Signal	(5a)	(5b)	(5c)	(1a)
6-Me	2.27	2.27	2.23	2.20
9-Me	2.54	2.55	2.29	2.36
1-Me	2.60	2.55	2.58	2.56
8-OMe			3.75	3.79
3-OMe			3.98	
7-CO_2Me	3.93	3.93	3.91	
2-H	6.67			
7-H				6.44
4-CHO	10.63	10.63	10.51	10.66
8-OH	11.34	11.35		
3-OH	12.08	12.73		12.77

of a hydrogen-bonded *o*-hydroxy-aldehyde grouping, and revealed the *o*-hydroxy-ester grouping to include a methoxycarbonyl entity. Signals assignable to three aryl methyl groups were also observed. Additionally, there also appeared in the spectrum of granulatin an aryl proton signal. That chlorogranulatin was the chlorinated analogue of granulatin was confirmed by the conversion of the latter substance to the former on chlorination in acetic acid.

In the mass spectrum of chlorogranulatin there appeared a cluster of fragments at *m/e* 211, 213, and



(3)
a; R = H, *m/e* 179
b; R = Cl, *m/e* 215,213

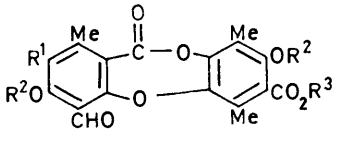


(4)
a; R = H, *m/e* 177
b; R = Cl, *m/e* 213, 211

215, shifted to *m/e* 177 and 179 in granulatin. Similar clusters of fragments, to which the ion structures (3) and (4) have been assigned, appear in the mass spectra of pannarin (1a) [3] and in agropsin (1b).[8] The pannarin-

type ring A structure is also characterized by ^{1}H n.m.r. signals at δ 2.56 (1-Me), 10.66 (4-CHO), and 12.77 (3-OH). Equivalent signals appear in the spectrum of chlorogranulatin (Table). Because of the foregoing spectral characteristics it was concluded that ring A of pannarin and chlorogranulatin were similarly substituted, and thus it followed that two aryl methyl groups and the *o*-hydroxy-methoxycarbonyl grouping were located in ring B of granulatin and chlorogranulatin. A more detailed mass spectral analysis (available as Supplementary Publication No. SUP 22492) * further substantiates these conclusions.

Methylation of chlorogranulatin yielded a dimethyl adduct for which the ^{1}H n.m.r. signals of the three aryl methyl groups occurred at differing chemical shifts. The signal at δ 2.58, a slightly broadened singlet ($W_{\frac{1}{2}}$ 1.3 Hz), can, by analogy with the pannarin studies,[3] be safely assigned to the 1-Me group. That the signals of the remaining two aryl methyl groups were broadened to a greater extent ($W_{\frac{1}{2}}$ 2.0 Hz), and indeed, when expanded, appeared as finely split doublets (J 0.5 Hz), indicated [3] a *para* relationship to exist between the ring B methyl groups, which must therefore be located at C-6 and C-9. Presumably the doublet patterns observed in this study, and in pannarin (1a) on decoupling of the 7-H proton signal, are in fact the inner legs of a long range quartet coupling existing between the C-6 and C-9 methyl groups. It therefore only remained to assign the positions of the ring B hydroxy and methoxycarbonyl groups. Biosynthetic considerations (see below) allow these groups to be confidently assigned to C-8 and C-7, respectively, hence the gross structures (5a), (5b), and (5c) were assigned to granulatin, chlorogranulatin, and its dimethyl adduct, respectively.



(5)

a; $R^1 = R^2 = H$, $R^3 = Me$
b; $R^1 = Cl$, $R^2 = H$, $R^3 = Me$
c, $R^1 = Cl$, $R^2 = R^3 = Me$
d; $R^1 = R^2 = R^3 = H$

Granulatin (5a) and chlorogranulatin (5b) are clearly the depsidone equivalents of the depsides atranorin (2a) and chloroatranorin (2b), respectively, and like the latter two substances the biosynthesis of the former two would appear to involve the condensation of two β-orsellinate units. This appears to be only the second † occasion on which depsidone metabolites of this type have been isolated. Aghoramurthy *et al.*[9] isolated from *Alectoria tortuosa* Merr. (syn. *A. virens* Tayl.) a metabolite designated virensic acid. Degradation studies indicated the substance to possess one of four possible structures. Structure (5d) was then advanced for virensic acid on the basis of a further consideration of i.r. spectral data. No other synthetic or spectral evidence has been subsequently presented in support of this structure. Since the physical constants of the methyl virensate appear to identify the ester prepared by Aghoramurthy *et al.* as granulatin, the additional spectral data now presented also serve to substantiate the assignment of a doubly condensed β-orsellinate structure to virensic acid.

EXPERIMENTAL

Melting points were determined on a Reichert Thermopan melting point instrument. Infrared spectra were determined for KBr discs on a Perkin-Elmer 180 instrument; ^{1}H n.m.r. spectra were determined for solutions in $CDCl_3$ at 60 MHz on a JEOL C-60HL spectrometer, and mass spectra were determined at 70 eV on a Varian M.A.T. CH5 instrument. Microanalyses were performed by Professor A. D. Campbell of the University of Otago, Dunedin.

Extraction of Pseudocyphellaria granulata.‡—The finely ground lichen material (15.4 g), collected in the vicinity of the Hollyford Junction, Fiordland, New Zealand, in August 1976, was extracted with light petroleum (b.p. 80—100°) (180 ml) in a Soxhlet apparatus for 48 h. Separation of the crystalline precipitate (210 mg) obtained on cooling, by preparative layer chromatography (p.l.c.) on silica gel with ether–light petroleum (b.p. 80—100°) (2 : 3) afforded in order of decreasing R_F, granulatin (5a) (25 mg), *chlorogranulatin* (5b) (112 mg), and hopane-6α,7β,22-triol (74 mg), m.p. and mixed [7] m.p. 226—228°. Further extraction of the lichen material with acetone (180 ml) yielded, on removal of the solvent, a residue (285 mg) consisting largely of hopane-6α,7β,22-triol, and the depsidones, stictic acid, norstictic acid, constictic acid, and salazinic acid.

Granulatin (5a) had m.p. 214—215° (from acetone) (lit.,[9] for methyl virensate 215—216.5°); $\nu_{max.}$ (KBr) 3 350—3 570 (OH), 1 745 (depsidone CO), 1 668 (*o*-hydroxyester), 1 650 (*o*-hydroxyaldehyde), 1 250—1 300, 1 205, 1 155, 1 079, 1 030, 970, 800, and 770 cm^{-1}; *m/e* 372 (M^+, 100%), 340 (61), 312 (77), 285 (37), 284 (27), 272 (81), 258 (68), 257 (14), 256 (24), 179 (15), and 177 (8) (Found: *m/e*, 372.083 8. Calc. for $^{12}C_{19}{}^1H_{16}{}^{16}O_8$: *M*, 372.084 5).

Chlorogranulatin (5b) had m.p. 202—203° (sublimed sample); $\nu_{max.}$ (KBr) 3 350—3 570 (OH), 1 739 (depsidone CO), 1 665 (*o*-hydroxyester), 1 648 (*o*-hydroxyaldehyde), 1 250—1 325, 1 205, 1 165, 1 124, 1 078, 1 030, 1 020, 970, 910, 900, 778, 771, 708, 641, and 600 cm^{-1}; *m/e* 408 (35%), 406 (M^+, 100%), 376 (21), 374 (55), 348 (29), 346 (73), 321 (10), 320 (13), 319 (31), 318 (26), 308 (24), 306 (65), 294 (32), 293 (18), 292 (95), 291 (10), 290 (18), 215 (5), 213 (16), and 211 (7) (Found: C, 56.2; H, 3.6; Cl, 9.2; *m/e*, 406.044 9. $C_{19}H_{15}ClO_8$ requires C, 56.1; H, 3.7; Cl, 8.8%. $^{12}C_{19}{}^1H_{15}{}^{35}Cl^{16}O_8$ requires *M*, 406.045 7).

Similar extraction of *P. faveolata* ‡ (13.2 g) with light

* For details of Supplementary Publications, see Notice to Authors No. 7 in *J.C.S. Perkin I*, 1978, Index issue.

† *Note added in proof:* The chlorinated depsidone described in this work clearly corresponds to that recently reported (W. S. Maass, A. G. McInnes, D. G. Smith, and A. Taylor, *Canad. J. Chem.*, 1977, **55**, 2839) from *Pseudocyphellaria physciopora* Nyl. The structure of this substance was independently elucidated (E. M. Goh, M.Sc. Thesis, University of Waikato, 1978) in our laboratory during 1977.

‡ We thank Messrs. D. J. Galloway and P. James, British Museum (Natural History), London, for the identification of the lichen materials. *P. faveolata* is considered to be the esorediate counterpart of *P. granulata*. Other chemical races of *P. faveolata* are known to contain different metabolites.

petroleum (b.p. 80—100°) (180 ml), afforded granulatin (5a) (20 mg), chlorogranulatin (5b) (108 mg), and hopane-6α,7β,22-triol (65 mg).

Chlorination of Granulatin.—A solution of granulatin (5a) (5 mg) in acetic acid (1 ml) was added to a solution of chlorine (2 mg) in acetic acid (0.5 ml), and the mixture stirred for 18 h at room temperature in the dark. Work-up and purification by p.l.c. on silica gel with ether–light petroleum (b.p. 80—100°) (1 : 1) as eluant gave chlorogranulatin (5b) (2.5 mg), *m/e* 408, 406, 376, 374, 348, 346, 321, 320, 319, 318, 308, 306, 294, 293, 292, 291, 290, 215, 213, and 211.

Methylation of Chlorogranulatin.—A solution of chlorogranulatin (5b) (50 mg) in methyl iodide (2 ml) and dimethylformamide (5 ml) was stirred over anhydrous potassium carbonate (2.5 g) for 18 h at room temperature. Work-up gave, as a semi-crystalline wax, *chlorogranulatin dimethyl ether* (5c) (33 mg) (Found: *m/e*, 434.977 5. $^{12}C_{21}{}^{1}H_{19}{}^{35}Cl^{16}O_8$ requires *M*, 434.076 9).

Repeated attempts to purify the crude reaction product by crystallization, or by p.l.c. on silica gel, were unsuccessful. Fractions recovered after p.l.c. and examined by ^1H n.m.r. spectroscopy were found to be complex mixtures, possibly including products arising from cleavage of the depsidone linkage.

We thank the University Grants Committee for the award of a research grant, the Fiordland and Urewera Natnaiol Parks Boards for assistance in the collection of lichen materials, the Nuffield Foundation for the award of a Research Scholarship (to A. L. W.), the keeper, British Museum (Natural History), London, for access to herbaria specimens, and Professor R. E. Corbett, University of Otago, Dunedin, for access to Dr. J. Murray's notes and specimens.

[8/1500 *Received*, 15*th August*, 1978]

REFERENCES

[1] S. Huneck, J. Redon, and W. Quilhot, *J. Hattori Bot. Lab.*, 1973, **37**, 539.
[2] I. Yosioka, *Yakagaku Zasshi*, 1941, **61**, 332.
[3] D. A. Jackman, M. V. Sargent, and J. A. Elix, *J.C.S. Perkin I*, 1975, 1979.
[4] J. Murray, deceased, unpublished notes.
[5] C. F. Culberson, 'Chemical and Botanical Guide to Lichen Products,' University of North Carolina Press, Chapel Hill, 1969.
[6] G. Koller and K. Popl, *Monatsh.*, 1934, **64**, 129.
[7] R. E. Corbett and A. L. Wilkins, *Austral. J. Chem.*, 1977, **30**, 2329.
[8] S. Huneck and I. M. Lamb, *Phytochemistry*, 1975, **14**, 1625.
[9] K. Aghoramurthy, K. G. Sarma, and T. R. Seshadri, *Tetrahedron*, 1961, **12**, 173.