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DEPSIDONE CONSTITUENTS FROM THE QUINTARIA GROUP OF *NEPHROMA* SPECIES

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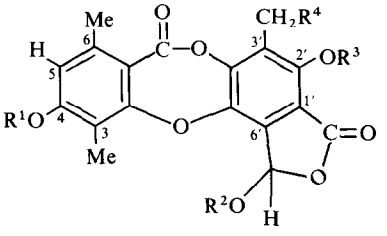
Key Word Index—*Nephroma antarcticum*; *N. australe*; Nephromaceae; lichens; β-orcinol depsidones; hypostictic acid; hyposalazinic acid; hypoconstictic acid.

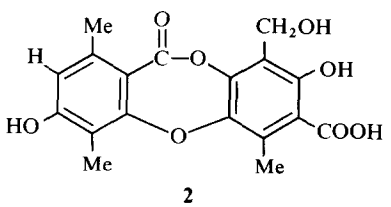
Abstract—A new lichen depsidone was isolated, in the form of its triacetate derivative from the acetylated extracts of *Nephroma antarcticum* and has been demonstrated to be hypoconstictic acid-triacetate. Two related depsidones, hypostictic acid and hyposalazinic acid, were isolated from *N. australe*.

INTRODUCTION

Hale [1, 2] has reported the presence in collections of *Xanthoparmelia quintaria*, *Pseudoparmelia neoquintaria*, *Relicina abstrusa*, and some brown *Parmelia* species, of four substances which Culberson [3] designated PQ-1, PQ-2, PQ-3 and PQ-4. Subsequently Keogh [4] reported some of these substances in a new species of *Thelotrema*, and showed PQ-1 and PQ-2 to be hypostictic acid (**1a**) and hyposalazinic acid [5] (**1b**), respectively. More recently Culberson has intimated to us in a personal communication that another of these substances (PQ-3) is identical with hypoprotocetraric acid (**2**), and that the structure of PQ-4 has not yet been defined.



1a R^1 = Me, R^2 = R^3 = R^4 = H
1b R^1 = R^2 = R^3 = R^4 = H
1c R^1 = Me, R^2 = R^3 = H, R^4 = OH
1d R^1 = Me, R^2 = R^3 = Ac, R^4 = H
1e R^1 = R^2 = R^3 = Ac, R^4 = H
1f R^1 = Me, R^2 = R^3 = Ac, R^4 = OAc
1g R^1 = Me, R^2 = R^3 = CD_3CO, R^4 = H



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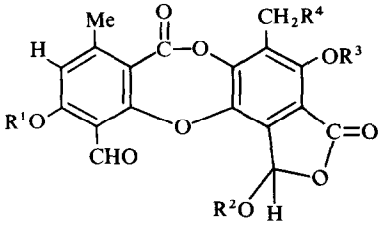


3a R^1 = Me, R^2 = R^3 = R^4 = H
3b R^1 = R^2 = R^3 = R^4 = H
3c R^1 = Me, R^2 = R^3 = H, R^4 = OH

RESULTS AND DISCUSSION

In the course of a chemotaxonomic survey of the genus *Nephroma* two of us (P. W. J. and A. L. W.) noted the presence of some of the foregoing substances in one of the two chemical races of *N. australe* (PQ-1 and PQ-2), and in *N. lobuligerum* and *N. antarcticum* (PQ-1 and PQ-4). Since the most polar of these substances (PQ-4) could not be satisfactorily isolated directly from the acetone extracts of *N. antarcticum*, which also contained hypostictic acid (**1a**), stictic acid (**3a**), constictic acid (**3c**), and traces of hyposalazinic acid (**1b**) and norstictic acid (**3b**), the extracts were acetylated and the major constituents were isolated as the corresponding acetates. By this means a modest quantity of PQ-4-triacetate was secured, and we now report spectral and synthetic correlations which reveal PQ-4-triacetate to be the 3-methyl analogue of constictic acid-triacetate. The designation hypoconstictic acid-triacetate is proposed for this compound.

A preliminary examination of the ^{1}H NMR spectra determined for hypostictic acid-diacetate (**1d**) and PQ-4-triacetate suggested the latter substance to differ from the former only to the extent that an aryl Me group signal at δ 2.32 had been replaced by aryl acetoxymethyl group signals at δ 5.30 ($\underline{CH_2}OAc$, ABq) and 1.97 ($CH_2\underline{OAc}$). This conclusion was also supported by the molecular formulations ($C_{23}H_{20}O_{10}$ and $C_{25}H_{22}O_{12}$) established for the respective substances, and by the presence, in the

mass spectra of the respective acetates, of fragment ions corresponding to the loss of up to two and three acetoxyl groups respectively. In each case the acetoxyl groups were lost mainly as ketene entities.

A total of five aryl Me group and acetate Me group signals appear in the ^{1}H NMR spectrum of hypostictic acid-diacetate (**1d**). On expansion, these signals were found to be of unequal half-band width and height. That the tallest and sharpest of these signals (δ 2.24 and 2.44) originated from the two acetoxyl groups was established by their absence from the ^{1}H NMR spectrum of the corresponding $^2D_6$-diacetate (**1g**).

Jackman *et al.* [6] and others [7, 8], have demonstrated that in depsidones such as granulatin and physciosporin a long range coupling of *ca* J = 0.5 Hz exists between a pair of aryl Me groups which are located *para* with respect to each other. Additionally, an *ortho*-aryl proton also couples [6] with one of the aryl Me groups and this results in a further broadening of the latter signal. In a series of decoupling experiments analogous to those described by Jackman *et al.* [6], we have demonstrated that similar couplings exist in the ring A portion of the hypostictic acid-diacetate molecule. For example, irradiation at δ 6.67 sharpened the signal at δ 2.53 (but not that at δ 2.17) and vice versa. The C-3 and C-6 Me group signals can therefore be assigned with confidence. The remaining Me group signal (δ 2.32) is of intermediate height and half-band width and must, by elimination, originate from the isolated C-3′ Me group.

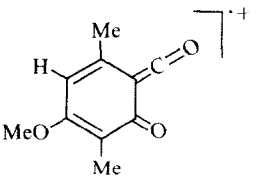
A similar analysis of the ^{1}H NMR spectrum determined for PQ-4-triacetate indicated the presence of an additional sharp acetate Me group signal at δ 1.97, and the absence of the C-3′ Me group signal. Signals corresponding to a pair of *para*-coupled aryl Me groups (one of which was coupled with an *ortho*-aryl proton) and two other acetate Me groups were also present. The chemical shift values of these signals, and also of the other lower field proton signals (see Table 1) correspond almost exactly to those determined for hypostictic acid-diacetate (**1d**). These observations suggested PQ-4-triacetate to be

Table 1. ^{1}H NMR assignments [δ (ppm) in $CDCl_3$]

Signal	Compound (1d)	(1f)	(1g)
3-Me	2.17 (1.8)	2.18 (1.8)	2.18 (1.8)
6-Me	2.53 (1.9)	2.56 (1.9)	2.53 (1.8)
3′-Me	2.31 (1.5)	—	2.32 (1.5)
3′-CH_2OAc	—	1.94 (1.1)	—
3′-CH_2OAc	—	5.30 (ABq)*	—
2′-OAc	2.24 (1.1)	2.25 (1.2)	—
6′-CH(OAc)O–	2.44 (1.0)	2.43 (1.1)	—
6′-CH(OAc)O–	7.51 (1.2)	7.50 (1.2)	7.50 (1.2)
4-OMe	3.91 (1.0)	3.92 (1.0)	3.91 (1.0)
5-H	6.67 (1.9)	6.67 (1.9)	6.67 (2.0)

* ABq, J = 11.5 Hz (doublets centred at δ 5.45 and 5.15).

Unless otherwise stated all signals are singlets, the half-band widths (in Hz) of which appear in parentheses after the chemical shift values.



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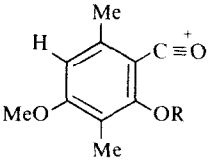

5a R = H
5b R = D

hypoconstictic acid-triacetate (**1f**). This conclusion is supported by the following observations. In both hypostictic acid-diacetate (**1d**) and PQ-4-triacetate the C-5 aryl proton signal resonates at δ 6.67. Thus, it can be inferred that a OMe group, rather than an acetoxyl group is located at C-4, since a OMe group typically shields an adjacent aryl proton to a greater extent (*ca* 0.21 ppm [7]). In hyposalazinic acid-triacetate the C-5 aryl proton signal appears [4] at δ 6.87. The structural significance of the ions of *m/e* 178 and 179 in the mass spectrum of 4-*O*-methylhypoprotocetraric acid and its fully methylated analogue have been discussed elsewhere [9]. Equivalent ions, to which structures **4** and **5a**, respectively, have been assigned [9], appear in the mass spectra of hypostictic acid-diacetate (**1d**) and of PQ-4-triacetate. In the case of hypostictic acid-$^2D_6$-diacetate (**1g**) the latter ion has *m/e* 180, hence, structure **5b** can be assigned.

Confirmation of the foregoing structural conclusions was obtained when a mixture of stictic acid (**3a**) and constictic acid (**3c**) was acetylated with acetic anhydride in pyridine, and subsequently hydrogenolysed over Pd–C to afford a mixture of hypostictic acid-diacetate (**1d**) and hypoconstictic acid-triacetate (**1f**). The selectivity of the acetylation reagent employed in this study is noteworthy in that the aldehyde group is not derivatized to a –$CH(OAc)_2$ entity, as is the case with, for example, acetic acid in the presence of sulphuric acid [10].

Since PQ-4 is more polar than either PQ-1 or PQ-2, it can be inferred that PQ-4 is hypoconstictic acid (**1c**).

EXPERIMENTAL

Nephroma australe was collected in December 1977 and May 1978 in the vicinity of Lakes Waikaremoana and Waikareiti, Urewera National Park, New Zealand. Fragments of *N. antarcticum* were detached from a collection in the Herbarium of the British Museum (Natural History) London (J. D. Hooker, Cape Horn).

Extraction of N. australe. The finely ground lichen material (4.8 g) was extracted in a Soxhlet apparatus with petrol for 17 hr and then with Me_2CO for 2.5 hr. The petrol extracts consisted largely of usnic acid and zeorin (hopane-6α,22-diol). Separation of the Me_2CO extracts (490 mg) by prep. TLC on Si gel with toluene–dioxane–HOAc acid (TDA) (90:25:4) gave two

fractions which were subjected to further prep. TLC on Si gel with petrol–Et_2O–HCO_2H (12:13:2) (PQ-1 fraction, faster moving band) or with TDA (PQ-2 fraction, slower moving band) to give hypostictic acid (**1a**) (PQ-1) (44 mg) and hyposalazinic acid (**1b**) (PQ-2) (16 mg), respectively.

Hypostictic acid (**1a**) had mp 260–262° with decomposition from 220° (lit. [4] 264° with decomposition); ν_{max}^{KBr} 1750, 1695, 1605 and 1560 cm^{-1}; δ 60 MHz (C_5D_5N) 2.43 (6-Me), 2.62 (3′-Me), 2.67 (3-Me), 3.83 (4-OMe), 6.82 (5-H), and 7.52 (6′C<u>H</u>(OH)O–); MS (probe) 70 eV *m/e* (rel. int.): 372 (M^+, 65), 354 (100), 328 (88), 327 (60), 326 (77), 300 (24), 299 (28), 298 (40), 272 (18), 271 (29), 270 (29), 244 (18), 243 (22), 242 (28), 216 (40) and 179 (21).

Hyposalazinic acid (**1b**) had mp 274° with decomposition from 219° (lit. [4] 280° with decomposition); ν_{max}^{KBr} 1720, 1695, 1610 and 1580 cm^{-1}; δ 60 MHz (C_5D_5N) 2.46 (6-Me), 2.63 (3′-Me), 2.81 (3-Me), 7.03 (5-H), and 7.93 (6′-C<u>H</u>(OH)O–); MS (probe) 70 eV *m/e* (rel. int.): 358 (M^+, 76), 340 (100), 314 (72), 313 (36), 312 (51), 286 (26), 285 (28), 284 (34), 258 (16), 257 (29), 256 (29), 230 (18), 229 (31), 228 (22) and 165 (68).

Hypostictic acid-diacetate (**1d**) *and hyposalazinic acid-triacetate* (**1e**). The Me_2CO extracts of *N. australe* (203 mg from 1.8 g of lichen) were dissolved in C_5H_5N–$(MeCO)_2O$ (1:1) (3 ml) and stirred for 16 hr at room temp. Work-up and purification by prep. TLC on Si gel with C_6H_6–Et_2O (93:7) gave hypostictic acid-diacetate (**1d**) (21 mg) mp 240° (lit. [4] 244°) and hyposalazinic acid-triacetate (**1e**) (8 mg) mp 201° (lit. [4] 203–205°).

Hypostictic acid-$^2D_6$*-diacetate* (**1g**). Repetition of the acetylation expt described above with a portion of the Me_2CO extracts from *N. australe* (170 mg from 1.46 g of lichen) and $(CD_3CO)_2O$ (0.5 ml) in C_5H_5N (2 ml) gave, as the major product, hypostictic acid-$^2D_6$-diacetate (1 g) (17 mg), MS (probe) 70 eV *m/e* (rel. int.): 462 (M^+, 15), 418 (100), 374 (72), 354 (96), 345 (16), 335 (23), 326 (71), 322 (33), 301 (25), 298 (36), 285 (31), 271 (39), 245 (23), 180 (14) and 178 (8).

Extraction of N. antarcticum. The finely ground lichen material (0.9 g) was extracted in a Soxhlet apparatus with petrol for 24 hr and then with Me_2CO for 6 hr. The petrol extracts consisted largely of usnic acid and zeorin. TLC (TDA) established the presence in the Me_2CO extracts of 3 major components, two of which gave distinctive red colourations when charred with H_2SO_4 [4]. Small scale expts established that the more polar of these substances could not be satisfactorily recovered from prep. TLC plates developed by the procedures employed for the separation of the *N. australe* extracts.

Reaction of the Me_2CO extracts (120 mg) with C_5H_5N–$(MeCO)_2O$ (1:1) (5 ml) for 24 hr at room temp. gave a gummy residue which was shown by TLC (petrol–Et_2O (3:2)) to consist of 3 major components, two of which gave red colourations when charred with H_2SO_4. Separation of the latter components by prep. TLC on Si gel with petrol–Et_2O (3:2) gave hypostictic acid-diacetate (**1d**) (22 mg) (higher R_f value). Hypoconstictic acid-triacetate (**1f**) had mp 190–192°; ν_{max}^{KBr} 1780, 1743, 1710, 1634, 1610, 1564, 1456, 1343, 1185, 1152, 1137, 1056, 986 and 913 cm^{-1}; MS (probe) 70 eV (rel. int.): *m/e* 514 (M^+, 10), 472 (15), 454 (11), 412 (40), 370 (100), 342 (32), 314 (19), 221 (24), 179 (18) and 178 (12). (Found: *m/e* 514.1129. $^{12}C_{25}$ $^1H_{22}$ $^{16}O_{12}$ requires: 514.1111.)

Synthesis of hypostictic acid-diacetate (**1d**) *and hypoconstictic acid-triacetate* (**1f**). A mixture of stictic acid (**3a**) and constictic acid (**3c**) (*ca* 1:1) (280 mg), isolated by co-crystallization from the Et_2O extract of *Pseudocyphellaria homeophylla* [11] was dissolved in a 1:1 soln of C_5H_5N–$(MeCO)_2O$ (10 ml) and stood for 2 hr at room temp. Work-up gave material which was demonstrated by ^1H NMR to be a mixture (*ca* 1:1) of stictic acid-diacetate, δ 60 MHz ($CDCl_3$) 2.22 (2′-OAc), 2.32 (3′-Me), 2.45 (6′-CH(O<u>Ac</u>)O–), 2.58 (6-Me), 4.05 (4-OMe), 6.88 (5-H), 7.40 (6′-C<u>H</u>(OAc)O–), and 10.47 (3-CHO) and constictic acid-triacetate, 1.95 (3′-CH_2O<u>Ac</u>), 2.25 (2′-OAc), 2.45 (6′-CH(O<u>Ac</u>)O–). 2.63 (6-Me), 4.05 (4-OMe), 5.45 (*d*), 5.15 (*d*), (3′-<u>C</u>H_2OAc, AB*q*, J = 11.5 Hz), 6.88 (5-H), 7.40 (6′-<u>CH</u>(OAc)O–), 10.47 (3-CHO).

Hydrogenolysis of the foregoing mixture over Pd-on-C prepared using the method of Keogh [4], afforded material which, when separated by prep. TLC on Si gel with C_6H_6–Et_2O (9:1) gave hypostictic acid-diacetate (50 mg) and hypoconstictic acid-triacetate (52 mg). The latter substance was identical with PQ-4-triacetate.

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