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PSEUDODEPSIDONES AND OTHER CONSTITUENTS FROM *XANTHOPARMELIA SCABROSA*

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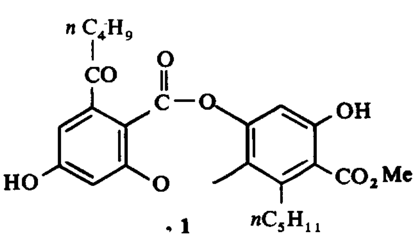
Key Word Index—*Xanthoparmelia scabrosa*; lichen; pseudodepsidones; norlobariol; norlobaridone; loxodin; loxodinol.

Abstract—The structural assignment of a new lichen constituent, loxodinol isolated from *X. scabrosa*, is described. Other constituents present were loxodin, norlobaridone, usnic acid and a pseudodepsidone norlabariol. The major sugar was mannitol, while the most abundant α-amino acids were aspartic and glutamic acids. A number of *X. scabrosa* samples from widely different geographical regions were screened and the presence or absence of loxodinol and norlobariol was successfully employed to distinguish between *X. scabrosa* and *X. mexicana*.

INTRODUCTION

The lichen genus *Xanthoparmelia* (Vainio) Hale comprises species primarily of rock or soil having usnic acid in the upper cortex (giving the plants their characteristic yellow-green colour), simple rhizines, and a pored epicortex external to the palisade plectenchyma of the upper cortex [1]. Fifteen species of *Xanthoparmelia* are recognized in the New Zealand flora [2] and chemistry plays an important part in their taxonomic separation. A characteristic feature of the chemistry of a group of predominantly Southern Hemisphere species within *Xanthoparmelia* is the presence of the structurally related secondary metabolites loxodin (1) and norlobaridone (2) [3, 4]. *X. scabrosa* (Tayl.) Hale is widely distributed in New Zealand, occurring in a wide range of habitats from coastal to subalpine localities. It is also an aggressive coloniser of man-made substrates such as bitumen, glass, slate and tiles [5]. In previous studies of some Australian materials referred to as *Parmelia conspersa* (Ehrh.) Ach., not only were the common metabolites usnic acid, salazinic acid and lobaric acid revealed [6], but also the rarer polyalicyclic nitrogen-containing compounds known collectively as scabrosins [7].



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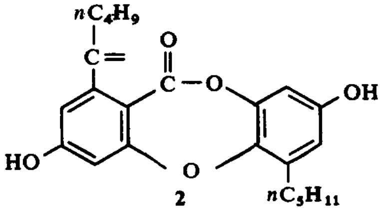

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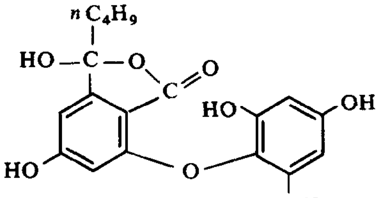
Most reports on lichen extractives concentrate on secondary aromatic metabolites since these often occur in abundance in the thallus and are also frequently of value in taxonomic studies [8–12]. In contrast, few studies concern themselves with other chemical components such as amino acids, polyhydric alcohols and sugars. The present report documents a study of the benzene-, acetone- and aqueous methanol-soluble fractions of *X. scabrosa*.

RESULTS AND DISCUSSION

The general extraction procedure adopted was successive Soxhlet extraction with benzene and acetone followed by cold extraction with aqueous methanol. The benzene extraction yielded a dark coloured solution containing loxodin, norlobaridone and usnic acid, the last-mentioned compound precipitating out upon concentration of the extract. These metabolites were identified using their mass spectra and TLC behaviour [13].

The acetone extract contained two phases, the lower phase solidifying on cooling. Dissolution of the solid in hot ethanol and subsequent cooling gave colourless needles, which were identified as mannitol. Glucose was also detected on the paper chromatogram of the crude solid. The acetone solution on concentration gave a gum. TLC revealed the presence of small amounts of loxodin and norlobaridone plus two unidentified components. The major one of these was assigned the structure of norlobariol (3) [14]. The minor compound which was isolated by preparative TLC and further purified by recrystallization from an acetone–water mixture gave a colourless solid which from mass spectroscopic data (M^+ 474) and elemental analysis was assigned the molecular composition $C_{25}H_{30}O_9$. The UV absorption showed maxima at 258, 295 and 306 nm indicating the compound to have depside- or depsidone-type structure. The ^1H



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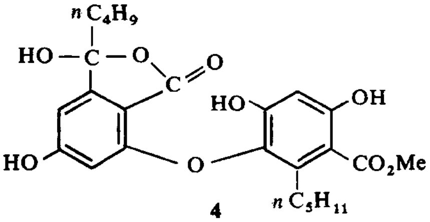
NMR spectrum is similar to that of loxodin and the identical chemical shifts and pattern of the signals at δ 6.62 (*d*, $J = 1.9$ Hz), 6.48 (*s*) and 6.05 (*d*, $J = 1.9$ Hz) and a singlet at 3.95 reflect the close structural similarity between the unknown compound and loxodin [3]. This was confirmed by alkali treatment of loxodin which generated the unknown plus some norlobaridone. The minor unknown compound also yielded some norlobaridone on treatment with alkali, although most remained unchanged. On the basis of these results, two probable structures could be proposed for the compound. The first structure may be indicated by the structure of loxodin existing as a free carboxylic acid through ring opening of the depside linkage. The alternative structure is that of the lactol compound, loxodinol (4).



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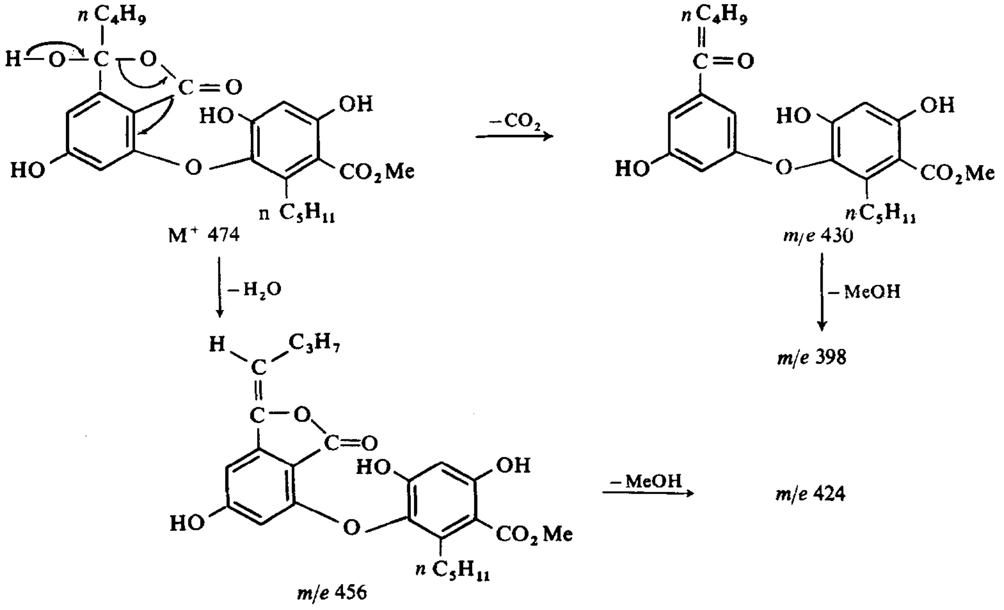
The IR spectrum of the compound showed no evidence of H-bonding which could be attributable to a carboxylic acid, and the carbonyl absorptions at 1700 and 1735 cm^{-1} are consistent with the ester and the lactol functions, respectively.

The proposed lactol structure of the compound was confirmed by the established formic acid reaction which caused dehydration resulting from the loss of the lactol hydroxyl and the adjacent proton [15]. With loxodinol, the same reaction yielded the expected dehydrated product, identified from mass spectroscopic data, which indicated a molecular ion at *m/e* 456. Further, treatment of loxodinol with diazomethane yielded a product whose MS was consistent with the methylation of three phenolic hydroxyls (M$^+$ 516).

The proposed fragmentation scheme (Scheme 1) for loxodinol not only accounts satisfactorily for the major peaks observed but also explains logically the observation that, when the probe temperature was increased, the intense mass peaks at *m/e* 474 and 456 greatly diminished while peak *m/e* 430 became more dominant.

The extractives of four of the eight samples of *Xanthoparmelia scabrosa* showed only trace amounts of loxodinol in spite of the presence of loxodin in comparable amount to the rest of the samples. This indicates that loxodinol is present naturally in the lichen and that there are two biological mechanisms operating on a common uncoupled phenol–acid intermediate, one of which produces the depsidone by the normal esterification process while the other produces the lactol via intramolecular nucleophilic addition of the carboxyl group to the *exo*-carbonyl function.

The lactol-type structures are not uncommon among lichen secondary metabolites. Stictic acid, norstictic acid, constictic acid and salazinic acid all have the five-membered lactol structure formed between the *ortho*-carboxyl group and the aldehyde function. With the exception of usnic acid, which is common in species of *Xanthoparmelia*, the proposed identities of norlobariol and loxodinol revealed the close structural relationship among the secondary aromatic metabolites of *X. scabrosa*. A survey made of eight samples of *Xantho-*



Scheme 1. Proposed fragmentation pattern for loxodinol.

Table 1. The distribution of some secondary metabolites in *Xanthoparmelia scabrosa* collected from different localities

Place of collection	Usnic acid	Salazinic acid	Norlobaridone	Loxodin	Norlobariol	Loxodinol
Lower Hutt	+ + +	+	+ + +	+ +	+ + +	+ +
East of Waiotapu Valley	+ + +	−	+ + +	+ +	+	+
West Matukituki Valley	+ + +	+ + + +	+ + +	+ +	−	+
Lake Rotoiti	+ + +	−	+ + +	+ +	+	+
Lamb Hill	+ + +	−	+ + +	+ +	+	+ +
Mt Wakins	+ + +	−	+ + +	+ +	+	+ +
Mt Peel	+ + +	+ +	+ + +	+ +	+ + +	+ +
Mangatua Summit	+ + +	+	+ + +	+ +	+	+

The number of + indicates increasing concentration.

parmelia scabrosa collected from widely different localities is shown in Table 1. The common feature of this particular taxon is the presence of major quantities of usnic acid, loxodin and norlobaridone. Loxodinol is also present in all the samples surveyed while norlobariol occurred in varying amounts from being completely absent in the sample from West Matukituki Valley to being the major constituent in samples collected from Mount Peel and Lower Hutt. Significant also is the observation that the sample from West Matukituki Valley contained salazinic acid as the major metabolite and this specimen has now been referred to the *X. mexicana* (Gyeln.) Hale. Salazinic acid was also detected in samples from Lower Hutt and Mt Peel, but was completely absent from the other samples.

This species, *X. mexicana* with a pale lower surface and simple to coralloid isidia which do not become sorediate, has as its major chemical compound salazinic acid in place of loxodin and norlobaridone. It has been rather sparsely collected in New Zealand and so far is known only from subalpine localities east of the Main Divide in the South Island. Although these collections have detectable amounts of loxodin and norlobaridone they lack norlobariol and loxodinol. Since collections of *X. scabrosa* contain varying (though small) amounts of salazinic acid, the presence of norlobariol and loxodinol in this taxon could, in combination with isidia morphology, serve to distinguish New Zealand populations of *X. scabrosa* and *X. mexicana*. Work on this question is continuing.

The methanol–water (4:1) extract containing ninhydrin-positive compounds was examined by 2D PC using *t*-butanol–acetic acid–water (3:1:1) and *t*-butanol–methyl ethyl ketone–ammonia–water (5:3:1:1) [16]. The following amino acids could be detected (the number of + indicates increasing concentration, approximated from the intensity of the colour): alanine (+ +), aspartic acid (+ + + +), glycine (+ +), glutamic acid (+ + + +), leucine (+), ornithine (+ +), serine (+ +), threonine (+ +), and valine (+). This distribution is comparable to those reported in other lichens [17–20].

EXPERIMENTAL

X. scabrosa was collected in April 1977 from the concrete kerb along a roadside in an industrial area of Lower Hutt. Thalli were soaked in cold water for several hr to remove soil and other foreign materials. The milled (air-dried) lichen (180 g) was sequentially extracted with boiling C_6H_6, and Me_2CO followed by cold extraction with 80% aq. MeOH. The C_6H_6 and Me_2CO extracts were worked up by PLC on Si gel G with C_6H_6–dioxane–HOAc (36:9:1) while the aq. MeOH extract was examined by 2D PC using *t*-BuOH–HOAc–H_2O (3:1:1) as the developing solvent. The amino acids were visualized with ninhydrin–cadmium spray [21].

Usnic acid. This compound separated out from the conc C_6H_6 extract. Recrystallization of the solid from EtOH gave yellow needles, mp 208°, having TLC and MS data identical with published data [13, 22].

Loxodin and norlobaridone. The conc C_6H_6 extract was fractionated by PLC to yield loxodin and norlobaridone. The more mobile fraction gave a solid, mp 132–136°, whose NMR and MS data were identical with published data on loxodin [3], while the slightly less mobile fraction was identical in all respects with authentic norlobaridone.

Mannitol and glucose. The cooled Me_2CO extract gave a solid which on crystallization from EtOH gave colourless needles, mp 158–162°. The PC and MS data were identical with those of authentic mannitol. PC of the crude solid from above also showed the presence of other compounds including a reducing sugar which was shown (by PC) to be glucose.

Norlobariol and loxodinol. The Me_2CO extract from above was concd and fractionated by PLC. The lower mobile fraction was the major component of this extract and was shown to be norlobariol [14]. The other lesser component at slightly higher R_f was further purified by recrystallization from Me_2CO–H_2O to give almost colourless needles, mp 235–237°. (Found: C, 62.8; H, 6.6. Calc. for $C_{25}H_{30}O_9$: C, 63.3; H, 6.3%). ^1H NMR (Me_2CO-d_6): δ 6.62 (1H, *d*, $J = 1.9$ Hz), 6.48 (1H, *s*), 6.05 (1H, *d*, $J = 1.9$ Hz), 3.95 (3H, *s*), 2.86 (>4H, *m*) probably due to presence of H_2O), 1.28 (10H, *m*) and 0.89 (6H, *m*). The compound showed UV absorption (EtOH) at 258, 295 and 306 nm while the MS showed peaks (intensity) at *m/e*: 474 (2%), 456 (20), 430 (35), 424 (20), 398 (100), 341 (20), 221 (30) and metastable ions at *ca* *m/e* 396, 368.5, 319 and 292.5. ν_{max}^{KBr} cm^{-1}: 3440(b), 1735, 1700.

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