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Secalonic Acid A from Pseudoparmelia sphaerospora (Nyl.) Hale and P. hypomilta (Fée) Hale (Parmeliaceae)

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Abstract. Secalonic acid A, a yellow pigment from fungal metabolism, was isolated from the lichens *Pseudoparmelia sphaerospora* and *P. hypomilta*. From *P. sphaerospora* was also isolated the depsidone hypostictic acid. The structure of these compounds was determined by spectroscopic methods and comparison with literature data.

Introduction

Lichens are known to produce substances considered specific to this plant group. However, some of these compounds have been found as well in fungi and vascular plants. Yosioka et al. (1968, 1971) isolated from *Parmelia entotheiochroa* Hue and *Cetraria ornata* Müll. Arg. the yellow pigments secalonic acid A and C, respectively. Seven isomers are known of secalonic acid: A, B, C, D, E, F and G, all isolated from fungi by Budavari (1989).

The genus *Pseudoparmelia* Lynge, as redefined by Hale (1986), encloses only four, tropical species, *P. cyphellata* Lynge, *P. chapadensis* (Lynge) Hale, *P. hypomilta* (Fée) Hale and *P. sphaerospora* (Nyl.) Hale. According to Hale (1986), all four species share an unidentified pale yellow medullary pigment, that is supposed to be secalonic acid, and rarely contain stictic or hypostictic acids in the medulla and traces of atranorin in the cortex. In the present paper results are presented of a chemical analysis of two species of this genus.

Experimental

The lichens were collected on bark in Vila Piraputanga, Mato Grosso do Sul, Brazil, in August, 1989. A voucher of each specimen was deposited in the herbarium of the Chemical Department of the Federal University of Mato Grosso do Sul. After having been cleansed the lichens were air-dried.

Pseudoparmelia sphaerospora - The powdered lichen (33,0 g) was extracted exhaustively with C_6H_6 followed by Me_2CO in a Soxhlet apparatus. The extracts were evaporated under reduced pressure.

Benzene extract - This extract was a mixture of three components. The first two were a yellow powder (fraction A) and a white powder present in minor proportion in this extract (fraction B). This latter fraction was the same component as present in the acetone extract. Beside these two major components, a third compound was present in trace amount and was identified by microcrystallization reaction in GAoT (glycerol:EtOH:o-toluidine 2:2:l) and by TLC on silica gel, as atranorin. The major component of this extract (fraction A) was isolated by prep. TLC on silica gel impregnated with 0.1 N oxalic acid and as solvent C_6H_6-EtOAc (5:3). The plates were sprayed with MeOH/sulfuric acid 10%. Fraction A (Rf 0.45) was eluated with Me_2CO and crystallized in $CHCl_3/Me_2CO$ as yellow needles, mp. 256-258° (uncorr.). IR (KBr) cm^{-1}: 3500 (OH), 1730 ($COOCH_3$), 1610 (C=O), 1585 and 1560 (aromatic). ^1H NMR (100 MHz DMSO-d_6, ppm): δ 1.04 (6H, d, J=6Hz, CH-CH_3 x 2), 2.30-2.73 (6H, m, C<u>H</u>-CH_3 x 2, -CH_2- x 2), 3.64 (6H, s, -$COOCH_3$ x 2), 3.80 (2H, d of d, J=6 and 10 Hz, C<u>H</u>OH x 2), 6.05 (2H, d, J=6Hz, CHO<u>H</u> x 2), 6.63 (2H, d, J=8 Hz, ArH x 2), 7.46 (2H, d, J=8 Hz, ArH x 2), 11.6 (2H, s, enolic OH), 13.6 (2H, s, phenolic OH). [Anal. found: C 60.11, H 4.74, Calc. for $C_{32}H_{30}O_{14}$ C 60.18, H 4.7].

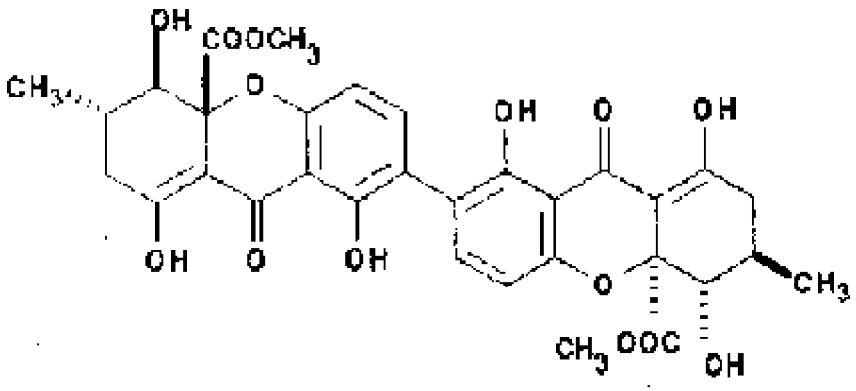
Acetonic extract - From this concentrated extract a compound (fraction B) that was separated by treatment with Me_2CO/H_20 (8:2), melted with decomposition at 287° (uncorr.). The TLC on silica gel using toluene-EtOAc-formic acid (138:89:8) as solvent mixture and sprayed with MeOH/sulfuric acid, showed a compound (Rf 0.42) that acquires a reddish colour. IR (KBr) cm^{-1}: 3410 (OH), 1755 and 1710 (lactone and depsidone, respectively), 1610 (C=O with intramolecular bond). ^1H NMR (60 MHz, DMSO-d_6, ppm.): δ 2.18 (3H, $ArCH_3$), 2.26 (3H, $ArCH_3$), 2.42 (3H, $ArCH_3$), 3.87 (3H, $ArOCH_3$), 6.68 (1H, d, J=8 Hz, ArH), 6.89 (1H, lactol) and 8.34 (1H, d, J=8 Hz, OH lactol).

Pseudoparmelia hypomilta - The powdered lichen (25.0 g) was exhaustively extracted with Me_2CO in a Soxhlet apparatus. The extracts were evaporated under reduced pressure, and the residue, after removal of pigments by EtOH, furnished a yellow-brown powder. Purification was carried out by preparative TLC on silica gel impregnated with 0.l N oxalic acid and as solvent mixture $CHCl_3$-MeOH (24:1). The spectral data (IR and ^1H NMR) of the isolated compound are identical with those present in secalonic acid A isolated from *P. sphaerospora*. After extraction with Me_2CO the powder of the lichen was treated with MeOH at room temperature during several days. The extracts were chromatographed on paper using C_6H_6-n.BuOH-pyridine-H_2O (1:5:3:3 upper phase) and EtOAc-HOAc-H_2O (6:3:2). The paper was sprayed with alkaline $AgNO_3$ and aniline phthalate. TLC of the MeOH extracts was developed with the second solvent and sprayed with p-anisaldehide-sulfuric acid. The chromatograms revealed the presence of mannitol and arabitol, identified by comparison with authentic samples. Mannitol was the major component.

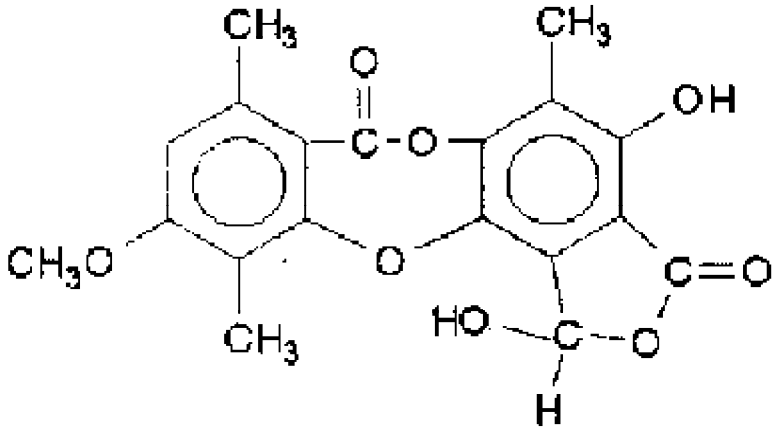
Results and discussion

The benzenic extract of *P. sphaerospora* showed by TLC on silica gel the presence of three components. One was isolated by preparative TLC (fraction A). The second was separated by treatment of the concentrated extract with Me_2CO (fraction B). This fraction is also the principal component present in the acetonic extract. In addition, we identified atranorin by microchemical methods and by chromatographic behaviour compared with an authentic sample.

Fraction A (yield 6.60%) was identified as secalonic acid A by spectral analysis (IR and NMR). Although the stereochemistry of fraction A was not determined, the integration of all protons and comparison of the chemical shifts with those reported by Andersen et al. (1977) for secalonic acid D, allowed us to establish for the compound isolated from *P. sphaerospora* the same structure



(1)



(2)

Fig. 1, 2: Structure of secalonic acid A (1) and hypostictic acid (2).

as this or its enantiomer secalonic acid A (Fig. 1). Since secalonic acid D is not known from lichens, while A is known from various lichens, the latter possibility seems the more likely.

Fraction B (yield 10%) was isolated from an acetonic extract. Its IR spectrum showed a sharp peak at 3410 cm^{-1}, characteristic of an OH group (non-bonded) and peaks at 1755 and 1710 cm^{-1} (lactone and depsidone, respectively), 1610 cm^{-1} (C=O with intramolecular bond) and 1560 cm^{-1} (aromatic). The NMR spectrum (DMSO-d_6) showed two signals at δ 2.l8 and 2.26 ppm, which represent two aromatic methyl groups, and one at higher field (δ 2.42 ppm), typical of an aromatic methyl group ortho to a C=O, a methoxy group (δ 3.87 ppm), and two protons each at δ 6.70 and 6.90 ppm. The latter signal at higher field is assigned to the proton geminal on the lactone ring of the depsidones stictic, norstictic, salazinic and constictic acid. A doublet at δ 8.34 ppm corresponds with the proton of the OH lactol group. These data are compatible with those cited in the literature for hypostictic acid (Keogh 1978). The chromatographic behaviour of fraction B is consistent with that described by Culberson (1972) and Culberson et al. (1981) for hypostictic acid (Fig. 2).

From *P. hypomilta* we isolated by preparative TLC a compound with the same characteristics as fraction A from *P. sphaerospora*. The IR and ^1H NMR spectra of this compound coincide with those of fraction A. This shows that secalonic acid A is the principal component present in *P. hypomilta* (yield 8%). In the methanolic extract of this lichen mannitol and arabitol were identified by chromatographic methods. Further minor compounds are present, but they were not studied due to the small concentration in the extracts. Atranorin was not detected in *P. hypomilta*.

Summarizing, we have isolated secalonic acid A as the principal compound of *P. hypomilta* and from *P. sphaerospora* we isolated secalonic acid A and hypostictic acid. Thus we were able to confirm the hypothesis of Hale (1986) that the pale yellow medullary pigment present in all species of the genus *Pseudoparmelia* is secalonic acid. This fact has an important chemotaxonomic significance for the identification of the genus *Pseudoparmelia*.

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