

Chemical Constituents of the Lichen *Stereocaulon azureum*

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Column chromatography of the acetone extract of the lichen *Stereocaulon azureum* afforded several substances identified by chemical and spectral means: α -amyrin, lupeol, taraxerol, ursolic acid, ergosterol peroxide, brassicasterol, cerevisterol, stictic acid and an acetone-condensation derivative, lobaric acid. Furthermore atranorin, methyl β -orsellinate, atranol and, for the first time from the genus *Stereocaulon*, cryptostictic acid and the dibenzofuran, strepsilin were isolated.

Introduction

Stereocaulon azureum (Schaer.) Nyl. (Stereocaulaceae, Lecanorales) is a silver-grey lichen with fruticose thallus, very ramified, graceful pseudopodia covered with squamulose or coralloidsquamulose phyllocladia, no soralia, frequent subterminal apothecia, dark-brown-blackish flat-to-convex disk. It is saxicolous and its preferred habitat in the Canaries is on the lava flow found on the "green mountainside" at 800 to 1000 m altitude where it may be very abundant locally. It has been reported as endemic to Madeira, the Azores and the Canary Islands (Lanzarote, Gran Canaria, Tenerife, Gomera, Hierro, and Palma).

The only chemical analysis of *S. azureum* to date has been by thin-layer chromatography for chemotaxonomic purposes. The most recent general study of *Stereocaulon*, by I. M. Lamb [1] characterized *S. azureum* chemotaxonomically as the only member of the genus in which lobaric and stictic acid are definitely known to co-exist and, for the first time, indicated that atranorin, norstictic acid and, possibly, constictic and consalazinic acids were also present. From 1858 until recently, *S. azureum* was classified as *Stereocaulon sphaerophoroides* Tuck [2].

In an intensive study of the numerous and varied Canary lichens, an Me₂CO extract of this intri-

guing species was analyzed and afforded various types of substances. Four triterpenes, α -amyrin, lupeol, taraxerol and ursolic acid, three sterols, ergosterol peroxide (1), brassicasterol (2), and cerevisterol (3) and various lichen compounds were obtained.

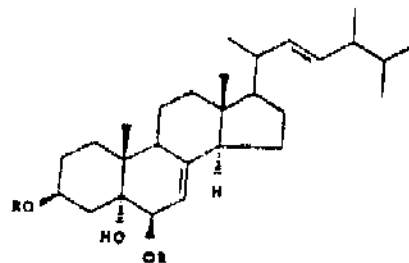
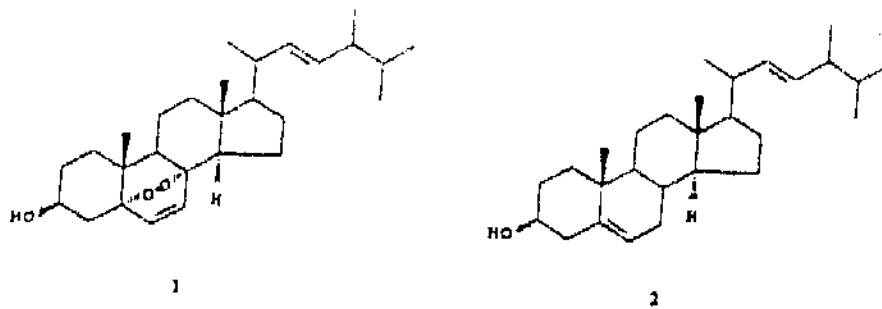
The major lichen substances were the depsidones stictic acid (4) and lobaric acid (5) and the depside atranorin, all three of which are of particular interest in the chemotaxonomic diagnosis of the species under study. An acetone-condensation artifact of stictic acid, 4a, two mono-aryl phenolic compounds, methyl β -orsellinate (7) and atranol (8) were also separated, as were the depsidone cryptostictic acid (4b) and the dibenzofuran strepsilin (9), found for the first time in a species of *Stereocaulon*.

Results and Discussion

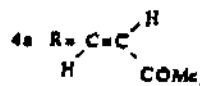
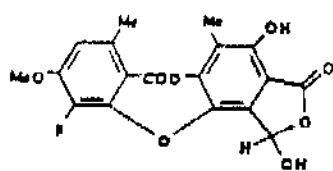
Isolation and structural determination of the terpenoids

A mixture of triterpenes was isolated from the fractions 8-11 of the column chromatography and later separated by silver nitrate-impregnated TLC into α -amyrin, lupeol and taraxerol, all originally isolated as lichen substances from *Cladonia macaronesica* [3].

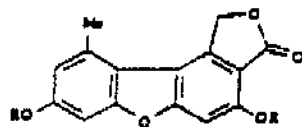
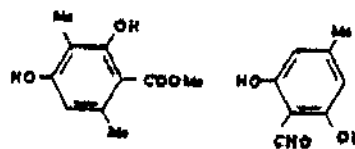
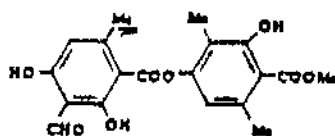
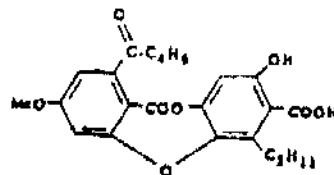
Fractions 17-19 yielded crystals with m.p. 146-147 °C, $[\alpha]_D^{25} -60^\circ$ and M^+ at m/z 398 for the molecular formula C₃₈H₆₆O. The ¹H NMR spectrum of this compound was very similar to that of ergosterol peroxide (1) with signals for four sec-



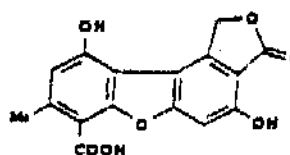
3a R = Ac



4b R = CH₂OH



9a R = Ac



ondary and three tertiary methyl groups, a signal for the geminal hydroxy proton as a multiplet at δ 3.51, which underwent a paramagnetic shift of approximately $\Delta \delta = 1$ ppm by acetylation, a two-proton multiplet for a double bond Δ^{22-23} , and a broad doublet at δ 5.38 assigned to another vinyl proton in the molecule. The above data exactly match brassicasterol (2) [4].

A triterpene acid and a sterol with the physical and spectroscopic data of ursolic acid [5] and ergosterol peroxide (1) [6], respectively, were isolated from the fractions 20–30.

Lastly, a sterol was obtained from the 51–58 fractions, and, when purified by chromatography on Sephadex LH-20 (hexane:chloroform:methanol, 2:1:1), formed colourless needles, m.p. 250–253 °C (from CHCl_3 :MeOH), M^+ at m/z 430, $\text{C}_{37}\text{H}_{66}\text{O}_2$. Its ^1H NMR spectrum greatly resembled that of ergosterol peroxide (1) with an extra geminal proton at δ 3.57 (1H, d, $J = 5.0$ Hz) coupled with a vinyl hydrogen at δ 5.29 ppm (1H, d, $J = 5.0$ Hz) sited at C-7. Treatment with Ac_2O and Py afforded a diacetylated derivative, 3a, with signals for a tertiary hydroxyl in its IR spectrum. These data agree with those of cerevisterol (3), earlier obtained as a lichen product from *Ramalina hirsutis* [7].

Isolation and structural determination of the lichen substances

The only depside isolated was obtained from the fractions 8–11 of the general column chromatography and its physical and spectral data agree with those reported for atranorin (6) [8, 9], a cortical substance present in most of the species of the genus *Stereocaulon*.

A mixture of depsidones was obtained from the fractions 39–58 and separated by chromatography on Sephadex LH-20 (hexane:chloroform:methanol, 2:1:1). A mixture of the similar compounds 4, 4a and 4b was obtained from the fractions 18–24 and separated by preparative chromatography on silica gel (Be:dioxan:HOAc). The ^1H NMR spectra of these three substances were alike except that a signal for an aldehyde group at δ 10.57 in 4 was replaced in 4a by those at δ 2.43 (3H, s) and 7.05 and 7.94 (each 1H, d, $J = 16.7$ Hz) for a radical ($-\text{CH}=\text{CH}-\text{COMe}$) as the result of a condensation reaction with acetone during the ex-

traction. In 4b, the same signal was replaced by those at δ 4.80 and 4.90 (each 1H, br s) for a ($-\text{CH}_2\text{OH}$) radical. The physical and spectroscopic data of 4, 4a and 4b proved to be identical to those of stictic acid (4), its acetone-condensation product, 4a, and cryptostictic acid (4b), earlier obtained from the lichen *Loharia pulmonaria* [10]. Cryptostictic acid (4b), a substance biogenetically related to 4, was isolated for the first time from the genus *Stereocaulon*. Another depsidone from the fractions 25–28 crystallized as colourless needles, m.p. 196–197 °C. Its MS showed the M^+ at m/z 456 in accordance with the empirical formula, $\text{C}_{22}\text{H}_{26}\text{O}_8$. Both the fragmentation pattern and the proton signals in MS and ^1H NMR coincided with those given in the literature for ioboric acid (5) [8, 9].

A dibenzofuran with colourless needles, m.p. 318–320 °C, was isolated from the fractions 31–38; its IR spectrum had absorption bands at 3463 and 3395 cm^{-1} (hydroxy groups) and 1736 cm^{-1} (carbonyl) and a typical aromatic band at 1626 cm^{-1} . MS showed the M^+ at m/z 270 for the empirical formula, $\text{C}_{17}\text{H}_{10}\text{O}_2$, and ^1H NMR gave signals for an aromatic methyl at δ 2.89, two meta aromatic protons at δ 6.94 and 7.12 (each 1H, br s), an aromatic proton at δ 7.34 (1H, s), and a two-proton singlet at δ 6.01 typical of a ($-\text{O}-\text{CH}_2-\text{Ar}$) benzylic methylene group. A diacetyl derivative, 9a (see Experimental for its MS and ^1H NMR spectra) was formed when the dibenzofuran was treated with Ac_2O -Py. All these data match strepsilin (9) [11], isolated here for the first time from the genus *Stereocaulon*. This is of especial interest as the substance usually isolated from this species is porphyritic acid (10), a dibenzofuran produced by a different coupling of the two phenol units [12].

One mono-arylic compound was isolated from the fractions 12–16 and another from the fractions 16–19 of the general chromatography and they were purified by chromatography on Sephadex LH-20 with 2:1:1 hexane:chloroform:methanol as eluant. Their physical and spectral data were those of methyl β -orsellinate (7) [13] and atranol (8) [14], respectively. These two substances, together with atranorin (6) had previously been isolated from various species of *Stereocaulon*. Caccumese *et al.* [14] have argued that 7 and 8 are found in *Stereocaulon vesuvianum* as true natural

products, dissenting from the earlier view that atranorin degradation during the extraction process probably accounted for these compounds [13]. Atranorin, which is probably involved in light harvesting and radiation protection, may fill another biological role in this context, being a depside with no intrinsic antibiotic activity, but able perhaps to serve as a storage compound which the plant utilizes in order to defend itself against attack by pathogen microorganisms, by slowly manufacturing the two bioactive compounds 7, a powerful fungicide, and 8, a strong antimicrobial agent. Norstictic, constictic and consalazinic acids [1] were not detected, but cryptostictic acid (4b), another biogenetic relative of stictic acid (4), was obtained for the first time ever from a *Stereocaulon*. Hence, it may be that the Canary Island species is a distinct chemotype.

Experimental

Melting points were obtained on a Kofler apparatus and are uncorrected. IR spectra were taken on a Perkin-Elmer Model 257 spectrophotometer. ^1H NMR were run at 200 MHz, and MS were obtained using a direct inlet system at 70 eV. The plant material was collected on January 29, 1989, on lava in the Biosphere Reserve "El Canal y los Tilos", at an altitude of 1300 m on the island of La Palma. Voucher specimens are held by the Herbarium TFC Lich. (No. 2033), Departamento de Biología Vegetal, Universidad de La Laguna.

Isolation of compounds

The dried lichen material was ground to give 696 g of a fine powder which was macerated in acetone. The acetone extract was concentrated at reduced pressure to give 26 g of a syrupy liquid which was again dissolved in acetone. A solid was precipitated which proved to be a mixture of at least three substances: stictic acid (4) (1 g), its condensation product, 4a (500 mg), and lobaric acid (5)

(2 g). The rest of the extract was passed through a column packed with 1 kg silica gel and eluted with mixtures of hexane-ethyl acetate of increasing polarity. Fractions of 500 ml were collected and monitored by TLC, giving the following: the fractions 1-7 (wax), the fractions 8-11 (α -amyrin + lupeol + taraxerol (50 mg), 6 (500 mg)); the fractions 12-16 [7 (600 mg)]; the fractions 17-19 [8 (350 mg), 2 (30 mg)]; the fractions 20-30 [ursolic acid (500 mg), 1 (30 mg)]; the fractions 31-38 [9 (10 mg)]; the fractions 39-50 [4 (100 mg), 4a (200 mg), 5 (500 mg)] and the fractions 51-58 [3 (25 mg), 4 (20 mg), 4a (30 mg), 4b (20 mg)].

Strepsilin (9): Colourless needles, m.p. 318-320 °C (CHCl₃-MeOH); IR ν_{max} (KBr) cm⁻¹: 3463, 3395, 1736, 1626; ^1H NMR (DMSO-d₆) δ : 2.89 (3H, s, Me-Ar), 6.01 (2H, s, -O-CH₂-Ar), 6.94 (1H, br s, H-Ar), 7.12 (1H, br s, H-Ar), 7.34 (1H, s, H-Ar), 9.70 (1H, s, OH-Ar); MS m/z (rel. int.): 270 [M]⁺ (100), 242 [M-CO]⁺ (22), 241 [M-CHO]⁺ (97), 213 (21), 185 (14), 155 (13), 149 (17), 127 (28).

Acetylation of 9: Compound 9 (5 mg) was treated with Ac₂O (0.5 ml) in pyridine (0.5 ml) and the mixture was left overnight at room temperature. Usual work-up gave a diacetyl derivative, 9a (5.5 mg); ^1H NMR (DMSO-d₆) δ : 2.33 (3H, s, OAc), 2.39 (3H, s, OAc), 2.83 (3H, s, Me-Ar), 6.02 (2H, s, -O-CH₂-Ar), 7.07 (1H, br s, H-Ar), 7.43 (1H, br s, H-Ar), 7.63 (1H, s, H-Ar); MS m/z (rel. int.): 354 [M]⁺ (1), 312 [M-C₂H₄O]⁺ (22), 270 [M-2 x C₂H₄O]⁺ (100), 242 [270-CO]⁺ (5), 241 [270-CHO]⁺ (30), 213 (8), 185 (6), 128 (5).

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