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STEROL AND OTHER CONSTITUENTS OF THE BROWN ALGA *DESMARESTIA ACULEATA*

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Key Word Index—*Desmarestia aculeata*; Desmarestiaceae; β-carotene; *trans*-phytol; plastoquinone-9; fucoxanthin; novel sterol.

Abstract—The brown alga *Desmarestia aculeata* was found to contain β-carotene, plastoquinone-9, fucoxanthin and a C_{27} sterol with a novel side chain.

INTRODUCTION

As part of a study of marine natural products from the Bay of Fundy, we have examined the constituents of the brown alga *Desmarestia aculeata* Lamouroux and we now report our findings.

RESULTS AND DISCUSSION

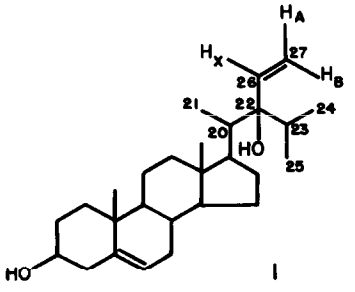
D. aculeata collected at Maces Bay, New Brunswick was extracted with methanol in a Soxhlet and the chloroform-soluble fraction of the extract subjected to column chromatography on silica gel. This resulted in the isolation of several known compounds and the novel sterol **1**.

The least polar compound, a red amorphous solid, proved to be β-carotene by comparison of spectral data [1]. The second compound eluted was separated from contaminating fatty acids by high-performance liquid chromatography (HPLC) and was isolated as an unstable yellow oil of MW 748 (MS). Examination of its IR and ^{1}H NMR spectra suggested it was plastoquinone-9 and this was confirmed by comparison with literature data [2]. The next compound, a viscous oil, gave a molecular ion *m/z* 296 in its mass spectrum. From its ^{1}H NMR spectrum it was identified as *trans*-phytol, the presence of a doublet (J = 7 Hz) at δ4.10 confirming the geometry of the double bond as *trans* [3]. Further elution of the column gave a mixture of triglycerides and a free sterol fraction, which on examination by gas chromatography (GC) proved to be a mixture containing at least 11 components. This fraction was followed by an oily, polar, unstable sterol which appeared homogeneous on silica gel TLC (MeOH–$CHCl_3$, 3:97 and EtOAc–hexane, 3:7) and was subjected to HPLC for final purification to furnish a crystalline, granular compound, mp 127–130°, whose structure **1** was deduced from spectral data. The 200 MHz ^{1}H NMR spectrum showed signals corresponding to four olefinic protons, three of which belonged to a tertiary vinyl group since they gave rise to an ABX system (see below) while the fourth appearing at δ5.36 was assigned to C-6H. Confirmation of the location of a double bond at C-5 was obtained by double irradiation experiments. Thus irradiation of the allylic proton region (~ δ2.3) caused the C-3H multiplet at δ3.50 to collapse to a broad doublet. The presence of two tertiary and three secondary methyl groups was substantiated by singlets at δ0.65 and 1.00 and doublets at δ0.84, 0.88 and 0.96 while the remaining protons lay in an envelope from δ1.2 to 2.3.

The mass spectrum displayed a base peak molecular ion at *m/z* 400 corresponding to $C_{27}H_{44}O_2$ and was supported by substantial fragments at *m/z* 385 $[M-Me]^+$, 382 $[M-H_2O]^+$, 367 $[M-Me-H_2O]^+$, 357 $[M-CHMe_2]^+$. The presence of the common cholesterol nucleus was attested to by the diagnostic [4–7] ions at *m/z* 271 and 314.

Thus we conclude that this new sterol possesses an eight-carbon side chain incorporating a secondary methyl group, an isopropyl moiety, a vinyl group and a tertiary alcohol as in structure **1**. Further study of this sterol was made difficult because of its instability and limited quantity. Examination of the 200 MHz ^{1}H NMR spectrum of its decomposition products indicated that the major components result from allylic rearrangement. Thus new multiplets centred at δ3.75 and 5.85 are associated with HO–CH_2–CH=C– methylene and methine features, respectively, while downfield shifts of δ0.25 are observed for the doublets due to 21-Me, 24-Me and 25-Me which are β to the side chain vinyl group in the possible geometrical isomers of the allylic rearrangement products. Traces of a decomposition product featuring a diene were suggested by a small multiplet centred at δ6.32. Thus the products of decomposition afford further support for the novel side chain features of this sterol.

Previous chemical studies on *D. aculeata* are limited to the hormonal secretions of its eggs and led to the identification of three monocyclic unsaturated hydrocarbons [8].



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EXPERIMENTAL

^{1}H NMR spectra were recorded using TMS as internal standard. Analytical and prep. TLC were performed with precoated silica gel G plates (Kieselgel 60, F-254). A Waters Associates HPLC unit equipped with a 440 absorbance and R401 refractive index detectors, employing a μ-Porasil 30 cm × 3.9 mm column was used for HPLC purifications.

Fresh *Desmarestia aculeata* (1.5 kg, wet wt), collected at Maces Bay, New Brunswick (November 1981), was finely chopped and immersed in MeOH and then extracted with MeOH in a Soxhlet for 48 hr. The extract was concd under red. pres. and the residue (30 g) dissolved in H_2O (300 ml) and extracted with $CHCl_3$ (3 × 500 ml). Evapn of the $CHCl_3$ extract yielded a brown solid (12 g) which was subjected to column chromatography on silica gel G (250 g), eluting with hexane, hexane–EtOAc, $CHCl_3$ and $CHCl_3$–MeOH in sequence. Fractions were examined by TLC, combined and purified by prep. TLC and HPLC. In order of elution, the following were obtained: β-carotene (0.07 g), plastoquinone-9 (0.01 g), *trans*-phytol (0.09 g), triglycerides (2.1 g), free sterols (0.10 g), sterol **1**, fucoxanthin (1.6 g).

Crude sterol **1** (0.037 g), a white solid, was purified by HPLC (5% MeOH in $CHCl_3$) and analysed by GC to provide pure sterol **1** (0.011 g) as granular crystals, mp 127–130°; IR ($CHCl_3$) cm^{-1}: 3500, 1450, 1320, 1150; ^{1}H NMR ($CDCl_3$): δ0.65 (*s*, 3H, 18-Me), 0.84 (*d*, 3H, J = 5 Hz, 24-Me), 0.88 (*d*, 3H, J = 5 Hz, 25-Me), 0.96 (*d*, 3H, J = 6 Hz, 21-Me), 1.00 (*s*, 3H, 19-Me), 3.51 (*m*, 1H, 3-H), 5.16 (*dd*, 1H, $J_{27A,27B}$ = 2, $J_{27A,26}$ = 11 Hz, 27-H_A), 5.28 (*dd*, 1H, $J_{27B,27A}$ = 2, $J_{27B,26}$ = 17 Hz, 27-H_B), 5.75 (*dd*, 1H, $J_{26,27A}$ = 11, $J_{26,27B}$ = 17 Hz, 26-H), 7.08 (*br s*, 1H, 30-H); MS, *m/z* (rel. int.): 400 $C_{27}H_{44}O_2$, $[M]^+$ (100), 385 $[M-Me]^+$ (22), 382 $[M-H_2O]^+$ (53), 367 $[M-H_2O-Me]^+$ (28), 357 $[M-Me_2CH]^+$ (11), 314 (42), 271 $[C_{19}H_{27}O]^+$ (14), 255 $[C_{19}H_{27}]^+$ (15), 231 $[C_{16}H_{23}O]^+$ (17), 213 $[C_{16}H_{21}]^+$ (39).

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CHAMAEJASMIN, A BIFLAVANONE FROM WOOD OF *DIPHYSA ROBINIOIDES*

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Key Word Index—*Diphysa robinioides*; Leguminosae; chamaejasmin; biflavanone; fungitoxin.

Abstract—The biflavanone chamaejasmin has been isolated from the wood of *Diphysa robinioides* and its structure established by the spectral data of the biflavanone and its hexaacetyl derivative.

Diphysa robinioides Benth is the only member of this tropical genus of the Leguminosae found in Costa Rica, where it occurs widely, and is commonly known as 'Guachipelín'. This tree can reach a height of 15 m, its wood is deep yellow in colour, very hard, resistant to attack by insects and to putrefaction. For this reason it is used in the construction industry where it may be exposed to a high degree of humidity. In Guatemalian folk medicine, this plant is reported to be useful in the treatment of cancer [1].

This communication describes the isolation and structural elucidation of the dimeric flavanone 5,7,4′,5″,7″,4‴-hexahydroxy-(3,3″)-biflavanone (**1**), from the wood of *Diphysa robinioides*. This biflavanone has been isolated only once before, from *Stellara chamaejasmine* (Thymeliaceae) [2].

Compound **1** is a crystalline product of mp 225–227° (MeOH–C_6H_6). Its ^{1}H NMR spectrum ($CDCl_3$, 100 MHz) exhibits very few signals but all of them constitute well-defined coupling systems which integrate