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Scalaradial Derivatives from the Nudibranch Chromodoris youngbleuthi and the Sponge Spongia oceania

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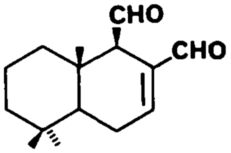
ABSTRACT -- Three deacetylscalaradials (3, 4, and 5) were isolated from the nudibranch Chromodoris youngbleuthi. Two of these (4 and 5) were absent among the constituents of the sponge Spongia oceania on which the nudibranch feeds. The structures were elucidated by spectral methods.

Sesterterpenes possessing the scalarane skeleton are known from marine sponges of the order Dictyoceratida and from a few dorid nudibranchs, which feed on these sponges.[1,2] Some of the scalaranes are ichthyotoxic and others exhibit antiinflammatory or cytotoxic activity in mammalian systems.[1,3,4] The vicinal dialdehyde function of the scalaradials suggested analogy with the well-known antifeedant sesquiterpene polygodial (1).[5] Two new deacetylscalaradials reported here were isolated from Chromodoris youngbleuthi along with epimers previously known from other dorid nudibranchs.[5] It is noteworthy that the major metabolites 4 and 5 from C. youngbleuthi are absent in its dietary sponge Spongia oceania.



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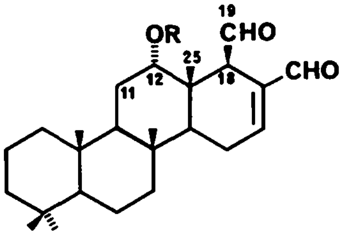

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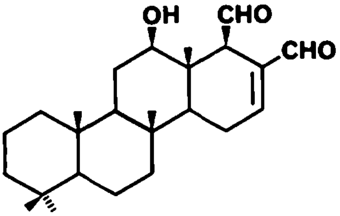

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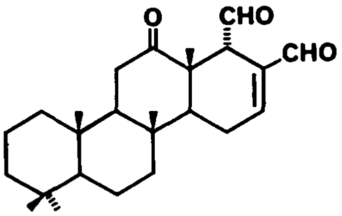

5

The dorid nudibranch *Chromodoris youngbleuthi* (*Chromolaichma youngbleuthi*)[6] is common in Hawaiian waters. It is unspectacular in appearance, tan colored and up to 200 mm long. The animals are frequently seen in caves and under ledges, at -5 to -10 m, feeding on *Spongia oceania*. We collected the nudibranch and the sponge near Pupukea on the north shore of O'ahu using SCUBA. The samples were transferred to the laboratory within hours of collection and extracted immediately. Whereas the nudibranchs were extracted rapidly with a methanol/dichloromethane mixture in order to isolate the skin extracts free of other metabolites, the sponge was steeped in dichloromethane overnight.

The NMR spectra of both crude extracts were indicative of mixtures of dialdehydes. These were initially separated on a BondElut silica column and subsequently by reversed phase HPLC. All metabolites displayed ^1H NMR spectral features characteristic of sesterterpenes. The two sponge metabolites were identified as scalaradial (2) and 12-deacetylscalaradial (3) on the basis of spectral comparison.[3] The nudibranch extract, on the other hand, contained no scalaradial, but three 12-deacetyl dialdehydes: the known 12-deacetylscalaradial (3), also present in the sponge, and two new compounds, 4 and 5, which were absent in *S. oceania*.

The ^1H NMR spectrum of 5 displayed its aldehydic proton signals at lower field (δ 10.75, 9.17) than did the other dialdehydes and lacked a signal assignable to the C-12 methine proton. The molecular formula of 5, $C_{25}H_{36}O_3$ (M^+ at *m/z* 384), indicated one additional degree of unsaturation by comparison with deacetylscalaradial. 2-D COSY, NOE and decoupling NMR experiments as well as proton splitting patterns identified the C-11 protons, which by their chemical shifts (δ 2.01 and 2.18) suggested the 12-oxo structure of 5. This was further confirmed by the presence of a third carbonyl resonance in the ^{13}C NMR spectrum at 212.6 ppm.

In polygodial (1) and scalaradial (2) the coupling constant (J = 2 Hz) of the unconjugated aldehyde proton is indicative of the stereochemistry at C-18, when the aldehyde group is axial.[7,8] 12-Deacetyl-18-*epi*-12-oxoscalaradial (5) was no exception: the C-19 proton signal appeared at 10.75 ppm as a doublet, J = 1.6 Hz. This was also confirmed by irradiation of the C-25 methyl protons, which caused an enhancement of the equatorial H-18 signal.

Although NMR data of the third dialdehyde (4) clearly marked it as a diastereomer of 12-deacetylscalaradial, its stereochemistry could not be elucidated by NOE studies alone. Irradiation of the C-25 methyl protons enhanced the signals due to H-19 and H-12, but not of the C-18 proton. In contrast to this, irradiation of H-12 caused enhancement of H-18. A possible explanation of these observations is that the NOE between the C-25 methyl protons and H-12 is in fact a secondary NOE resulting from a three-spin transfer via the C-11 protons.[9] Conclusive assignment of the 12-deacetyl-12-*epi*scalaradial structure (4) was made after acetylation and comparison of spectral features with published data.[3]

The dialdehyde moiety in the polygodials is well documented as the source of biological activity.[10] Both the spicy taste and the antifeedant activity of polygodial are attributed to the equatorial aldehyde which is close to the enal functionality.[11] The apparent lack of spicy taste in the scalaradials was suggested to be due to the larger size of the molecule.[5,12] However, both 12-deacetylscalaradial (3) and 12-deacetyl-12-*epi*scalaradial (4) taste distinctly more bitter (to B.T.) than 12-deacetyl-18-*epi*-12-oxoscalaradial (5). Fish feeding studies on *Tilapia* sp. in freshwater failed to quantify this finding. All dialdehydes seemed to be equally palatable to *Tilapia* which were fed food pellets treated with known concentrations of the metabolites. On the other hand, preliminary field experiments on filefishes showed on the basis of frequency counts that deacetylscalaradial was rejected more frequently than 12-deacetyl-18-*epi*-12-oxoscalaradial.

The relative amounts of the dialdehydes (4 > 5 > 3) in *C. youngbleuthi*, may suggest that 12-deacetylscalaradial (3) is converted into the other metabolites by the nudibranch. Absence of 4 and 5 in the diet source supports this view. Earlier evidence has shown in one case that a dorid nudibranch is capable of *de novo* terpene synthesis.[13] Formation of the 18-epimer is not too surprising since this would only require mildly basic conditions as evidenced in the polygodial series.[14] However, epimerization at C-12 appears to be a two-step process: oxidation to 5 followed by stereospecific reduction to 4. Since 4 is the most abundant metabolite, it perhaps is the one that is ecologically significant.

EXPERIMENTAL

NMR spectra were recorded on a Nicolet NT-300 instrument. Ultraviolet spectra were measured on a Beckman DU-7 spectrophotometer. Infrared spectra were recorded on a Nicolet FT instrument. Mass spectra were recorded on a Varian MAT-311 electron impact spectrometer. Optical rotations were measured on a Rudolph Research Autopol II polarimeter using a 5 cm micro cell.

Collection and Isolation:

Chromodoris youngbleuthi. Seventy-five specimens were collected at Pupukea, O'ahu in July, 1984. The collections were made at -10 m using SCUBA. The live animals were steeped in MeOH/CH_2Cl_2, 1:1, for 15 min. Soaking the animals a second and third time for extended periods did not yield further amounts of metabolites. The extract was evaporated giving a pale yellow solid (70 mg). The crude mixture was passed through a BondElut silica column using a gradient system from hexane to EtOAc and subsequently separated on HPLC (reversed phase C-18, CH_3CN/H_2O, 9:1) to obtain 12-deacetylscalaradial (3, 4 mg, 0.05 mg/animal), 12-deacetyl-12-*epi*scalaradial (4, 12 mg, 0.16 mg/animal), and 12-deacetyl-18-*epi*-12-oxoscalaradial (5, 9 mg, 0.12 mg/animal).

Spongia oceania. The sponge (630 g wet weight) was collected at locations where *C. youngbleuthi* were grazing. The homogenized sponge was steeped in CH_2Cl_2 overnight. The extract was evaporated giving a gray solid and processed as described above.

12-Deacetyl-12-episcalaradial (4): mp 201-203°C; $[\alpha]_D$ +38.1° (*c* 0.21, CH_2Cl_2); UV (EtOH): λ_{max} 230 nm; IR ($CHCl_3$): ν 3470, 1712, 1685 cm^{-1}; ^1H NMR ($CDCl_3$): δ 9.76 (d, 1H *J* = 3.5 Hz), 9.39 (s, 1H), 7.07 (br t, 1H), 3.72 (dd, 1H *J* - 11.2, 2.1 Hz), 3.12 (br d, 1H), 0.93 (s, 3H), 0.87 (s, 3H), 0.82 (br s, 6H), 0.79 (s, 3H); MS: *m/z* 386 (M^+).

12-Deacetyl-18-epi-12-oxoscalaradial (5): mp 179-182°C; $[\alpha]_D$ +120° (*c* 0.25, CH_2Cl_2); UV (EtOH): λ_{max} 232 nm; IR ($CHCl_3$): ν 1730, 1685 cm^{-1}; ^1H NMR (C_6D_6): δ 10.75 (d, 1H *J* = 1.6 Hz); 9.17 (s, 1H), 6.04 (br t, 1H *J* = 2.70), 3.69 (m, 1H), 2.18 (dd, 1H *J* = 13.87, 2.36 Hz), 2.01 (t, 1H *J* = 14.06 Hz), 0.89 (s, 3H), 0.84 (s, 3H), 0.76 (s, 3H), 0.60 (s, 3H), 0.56 (s, 3H); ^{13}C NMR (C_6D_6): δ 212.6, 211.1, 191.5, 148.8, 142.0, 58.5, 56.1, 54.6, 54.0, 53.3, 42.0, 41.0, 39.2, 37.8, 37.2, 35.1, 33.4, 33.3, 23.0, 21.4, 18.6, 18.1, 16.2, 15.7, 15.2; HRMS: *m/z* 384.2632 ($C_{25}H_{36}O_3$ requires 384.2665).

Acetylation of 12-Deacetyl-12-episcalaradial (4): To a dichloromethane solution of the dialdehyde (3.9 mg, 0.010 mmol) stirred at 0°C, acetic anhydride (1.3 mg, 0.013 mmol), triethylamine (0.7 mg, 0.012 mmol) and 4-*N*,*N*-dimethylaminopyridine (0.3 mg, 0.003 mmol), each dissolved in dichloromethane (0.5 mL), was added dropwise. The mixture was stirred at 0°C for 1 h. and at RT for another hour. After extraction with 0.2 N HCl and water the dichloromethane solution was passed through a BondElut Silica column to give a white solid after evaporation of the solvent homogenous on TLC (3 mg, 69%). Further purification on HPLC (reversed phase C-18, CH_3CN/H_2O, 9:1) gave 12-*epi*scalaradial with identical spectral characteristics to those reported.[3]

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