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OCCURRENCE OF OLEPUPUANE IN TWO MEDITERRANEAN NUDIBRANCHS: A PROTECTED FORM OF POLYGODIAL

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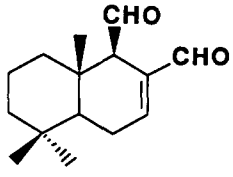
Since the discovery of polygodial [**1**] as the principal biologically active component in the defensive secretion of *Dendrodoris limbata* Cuvier (Nudibranchia, Doridacea) (1,2) and other dorid nudibranchs (3,4), efforts have been made to understand how such animals manage to avoid suffering from the effects of their own chemical weapon (5). Polygodial has been shown to be toxic to *D. limbata* when injected directly into the hepatopancreas (5), probably due to its marked reactivity with primary amino groups (6,7).

Extraction of the mantles of *D. limbata* and *Dendrodoris grandiflora* von Rapp under carefully controlled conditions and rapid isolation of the metabolites show that substantial amounts of the related (3) sesquiterpene olepupuane [**2**] are contained in these nudibranchs and that **2** is likely transformed into **1** during extraction and isolation procedures.



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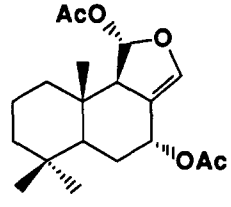
EXPERIMENTAL

The mantles of *D. limbata* (15 specimens) and *D. grandiflora* (5 specimens) were extracted as previously reported (1,4), except the Et_2O extracts were carefully dried over Na_2SO_4 before evaporation of the solvent. Voucher specimens are available for inspection at the Istituto per la Chimica di Molecole di Interesse Biologico. Tlc analysis [C_6H_6-Et_2O (9:1)] revealed the presence of a metabolite (R_f 0.55) moving faster than polygodial (R_f 0.40). This metabolite was shown later to be olepupuane [**2**] and was not detected when the Et_2O extracts were not dried. The approximate ratio between the metabolite R_f 0.55 and polygodial was ca. 1:1 in *D. limbata* and 1.5:1 in *D. grandiflora* as judged from the relative intensity of the spots in the tlc of the crude extracts, after spraying the tlc plates with $Ce(SO_4)_2$-H_2SO_4 and warming.

The ethereal extract of *D. limbata* was chromatographed on a flash Si gel column [*n*-hexane-EtOAc (85:15)] to afford nearly pure olepupuane [**2**] (20 mg) and polygodial [**1**] (90 mg) contaminated by a small amount of sterols.

Olepupuane [**2**] was identified by its ^{1}H-nmr properties: δ ($CDCl_3$) 6.38 (two overlapping doublets, 2H, J = 2.3 and 1.8 Hz), 5.65 (m, 1H), 2.60 (m, 1H), 2.09 (s, 3H), 2.06 (s, 3H), 0.86 (s, 3H), 0.82 (s, 3H), 0.79 (s, 3H); δ (C_6D_6) 6.70 (d, 1H, J = 2.3 Hz), 6.14 (d, 1H, J = 2.3 Hz), 5.65 (m, 1H), 2.73 (m, 1H), 1.65 (s, 3H), 1.58 (s, 3H), 0.77 (s, 3H), 0.63 (s, 3H), 0.56 (s, 3H). [The ^{1}H-nmr data of **2** were incompletely reported by Okuda *et al.* (3). In addition, the overlapping protons at δ 6.38 in the $CDCl_3$ spectrum were incorrectly reported at δ 6.53.



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The identity of olepupuane was secured by comparison of the C_6D_6 ^{1}H-nmr spectrum with that kindly provided by Dr. D.J. Faulkner.]

To ascertain whether prolonged contact on Si gel transforms **2** into **1**, a pure sample of olepupuane [**2**] (5 mg) was applied to two analytical Si gel plates (20 × 20 cm) and eluted with C_6H_6-Et_2O (9:1). Under a shortwave uv lamp the presence of three bands on each plate was ascertained; two major bands had R_f values corresponding to olepupuane and polygodial while a third minor band displayed an intermediate R_f. The three bands were eluted separately with

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Et_2O, and all were shown by the tlc and ^1H nmr to consist of polygodial. The nature of the intermediate minor band, originating from olepupuane and giving rise to polygodial on contact with Si gel, is unknown at present.

DISCUSSION

The results show that olepupuane [**2**] is transformed into polygodial [**1**] either during extraction procedures when the extracts are not dried or during chromatographic purification on Si gel. Since the transformation of **2** into **1** is a very easy reaction requiring only H_2O and trace amounts of acid (3), it cannot be assessed whether all the polygodial occurring in *D. limbata* and *D. grandiflora* originates during isolation. In a previous study (3) involving five porostome nudibranchs, mixtures of polygodial and olepupuane were found in three species while the remaining two species were reported to contain only olepupuane.

It would be reasonable to presume that polygodial may not be originally present in the nudibranchs in a free state, because it has a marked chemical reactivity towards primary amino groups (6) and could cause extensive damage of the nudibranch proteins. Therefore, olepupuane could act as an easily stored, masked form of polygodial that is transformed into the potent antifeedant when contact with predators becomes evident. Olepupuane was reported (3) to have antifeedant properties comparable to those of polygodial. However, it cannot be said whether the experimental conditions used for the assay converted olepupuane to polygodial in situ.

Protected forms of 1,4-dialdehydes are contained in other opisthobranch molluscs (8,9) as well as in tropical marine algae (10) that display a herbivore deterrent role. Also, basidiomycetes belonging to several genera, particularly *Lactarius* and *Russula*, store fatty acid esters of velutinal, which are transformed into the biologically active dialdehydes during extraction (11), and an isolate from the extracts of the plant *Linaria saxatilis* is transformed into the dialdehyde isolinaridial during chromatographic purification (12).

ACKNOWLEDGMENTS

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LITERATURE CITED

1. G. Cimino, S. De Rosa, S. De Stefano, and G. Sodano, *Comp. Biochem. Physiol,* **73B**, 471 (1982).
2. G. Cimino, S. De Rosa,, S. De Stefano, G. Sodano, and G. Villani, *Science,* **219**, 1237 (1983).
3. R.K. Okuda, P.J. Scheuer, J.E. Hochlowski, R.P. Walker, and D.J. Faulkner, *J. Org. Chem.*, **48**, 1866 (1983).
4. G. Cimino, S. De Rosa, S. De Stefano, R. Morrone, and G. Sodano, *Tetrahedron,* **41**, 1093 (1985).
5. G. Cimino, S. De Rosa, S. De Stefano, and G. Sodano, *Experientia,* **41**, 1335 (1985).
6. G. Cimino, G. Sodano, and A. Spinella, *Tetrahedron,* **43**, 5701 (1987).
7. V. Caprioli, G. Cimino, R. Colle, M. Gavagnin, G. Sodano, and A. Spinella, *J. Nat. Prod.*, **50**, 146 (1987).
8. C. Ireland and D.J. Faulkner, *Bioorg. Chem.*, **7**, 125 (1978).
9. G.R. Schulte and P.J. Scheuer, *Tetrahedron,* **38**, 1857 (1982).
10. V.J. Paul and W. Fenical, in: "Biorganic Marine Chemistry." Ed. by P.J. Scheuer, Vol. 1, Springer-Verlag, Berlin-Heidelberg, 1987, p. 1.
11. O. Sterner, R. Bergman, J. Kihlberg, and B. Wickberg, *J. Nat. Prod.*, **48**, 279 (1985).
12. A. San Feliciano, A.F. Barrero, J.M. Miguel del Corral, M. Gordaliza, and M. Medarde, *Tetrahedron,* **41**, 671 (1985).

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