

THE ISOLATION OF LOLIOLIDE FROM AN INDIAN OCEAN OPISTHOBRANCH MOLLUSC¹

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In 1938 Manske reported (2) a neutral substance obtained from *Fumaria officinalis* (Papaveraceae) that was eventually shown to be the monoterpene loliolide isolated from the perennial ryegrass *Lolium perenne* (Graminae) and nicely assigned structure I by Hodges in 1964 (3). Subsequently loliolide has been identified in 14 other higher plant species representing a total of 13 families (table 1,

variety of related substances (17). Similarly, photolysis of the zeaxanthin diepoxide (violaxanthin) from yellow pansies (*Viola tricolor*) yields a mixture containing loliolide (18). Both zeaxanthin and violaxanthin are ubiquitous carotenoid pigments and may certainly account, in part, for loliolide formation in some plant species. Furthermore, even mild nickel (IV) oxide oxidation of flavoxanthin and chry-

TABLE 1. Isolation of loliolide from terrestrial plants.

Plant	Family	Reference
<i>Acnistus arborescens</i>	Solanaceae	(4)
<i>Arnica montana</i>	Compositae	(5)
<i>Canscora decussata</i>	Gentianaceae	(6)
<i>Cocculus carolinus</i>	Menispermaceae	(7)
<i>Cypholophus decipiens</i>	Urticaceae	(8)
<i>Digitalis lanata</i>	Scrophulariaceae	(3)
<i>Digitalis purpurea</i>	Scrophulariaceae	(3, 9)
<i>Fumaria officinalis</i>	Papaveraceae	(2)
<i>Lolium perenne</i>	Graminae	(3)
<i>Lythrum salicaria</i>	Lythraceae	(10)
<i>Menyanthes trifoliata</i>	Menyanthaceae	(11)
<i>Nicandra physaloides</i>	Solanaceae	(12)
<i>Plantago lanceolata</i>	Plantaginaceae	(13)
<i>Plantago major</i>	Plantaginaceae	(14)
<i>Tripetaleia paniculata</i>	Ericaceae	(15)
<i>Undaria pinnatifida</i>	Laminariaceae	(16)

4-16). The occurrence of loliolide in higher plants appears on the way to becoming fairly common. Presumably loliolide arises as an oxidation product of certain plant carotenoids (6).

The photochemical oxidation of zeaxanthin (2) (the yellow pigment of corn, and the petals of *Physalis*) has been found to produce loliolide and a

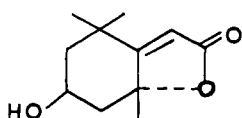
santhemaxanthin from the flowerheads of *Taraxacum officinale* (Compositae) was found sufficient to afford loliolide in 25% yield (19). Such experiments suggest that loliolide might be formed by an oxidative reaction of such carotenoids during isolation. However, there is evidence (6) that loliolide may be a normal plant metabolite. The roots of *Canscora decussata* (Gentianaceae) found to be free of carotenoid pigments still yielded lolio-

¹Contribution 69 in the series Antineoplastic Agents. For part 68 refer to (1).

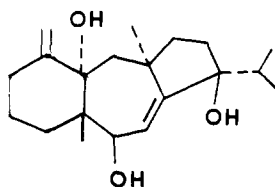
lide. As pointed out by Ghosal and colleagues (6), loliolide may be a normal metabolite of violaxanthin, known to occur in the flowers and fruit of this species, and may move to the roots during growth.

The photochemical conversion of zeaxanthin to loliolide allowed assignment of the absolute configuration (17), and a circular dichroism study substantiated the configurational orientation (20). A variety of total syntheses of (=)-loliolide (21, 22, 23, 24) and a recent synthesis of (-)-loliolide (25) have amply confirmed the skeletal

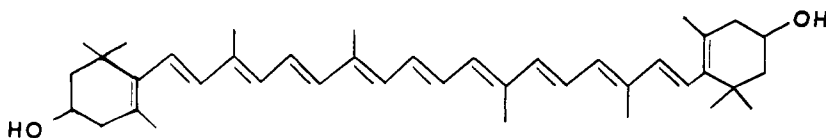
Initially *D. ecaudata* was collected in the Western Indian Ocean in 1972; the recollection used in the present study was obtained in the fall of 1976. An ambient temperature ethyl alcohol extract was separated by a series of solvent partition steps to provide a chloroform soluble fraction. Careful chromatographic separation of this mixture with silica gel provided both dolatriol² (3), which we previously isolated from *Dolabella auricularia* (26), and a fraction containing (-)-loliolide. Further separation of the latter fraction by partition chromatography on



Loliolide 1



Dolatriol 3



Zeaxanthin 2

and configurational relationships assigned this substance. Furthermore, some of the more recent references pertaining to loliolide contain spectral data (infrared to ¹³C-nmr) leading to the same general conclusions. In short, loliolide is a well characterized higher plant constituent that seems to have escaped previous biological evaluation.

As part of our detailed investigation (26, 27) of opisthobranch molluscs as new sources of potentially useful cancer chemotherapeutic drugs, a study of the Indian Ocean *Dolabella ecaudata* was undertaken. One of the cytotoxic constituents of this green sea hare was found to be (-)-loliolide.

Sephadex LH-20 provided crystalline (-)-loliolide. Originally some 10 milligrams of this substance was obtained and the structure was ascertained by a combination of mass spectral, infrared, and proton (and carbon-13) nuclear magnetic resonance measurements. Comparison with an authentic specimen of (-)-loliolide³ confirmed the structural assignment. The isolation of (-)-loliolide from *Dolabella ecaudata* represents, to our knowledge, the first discovery of this substance in animal tissue. Perhaps (-)-loliolide is not a

²A series of other new diterpenes have been isolated from *Dolabella californica* (28, 29).

³We wish to thank Professor R. Hodges for generously providing this sample.

natural constituent of *Dolabella ecaudata* but, rather, a carotenoid artifact produced during isolation or a dietary (algal) by-product. A definitive answer to this question will have to await results of experiments specifically designed to assess the matter.

An evaluation of (-)-loliolide against cell lines from the National Cancer Institute's human nasopharynx carcinoma (KB) and murine lymphocytic leukemia (P388) (30) gave cell growth inhibition values (ED_{50}) of 10 $\mu\text{g}/\text{ml}$ and 3.5–22 $\mu\text{g}/\text{ml}$, respectively. However, at doses of 2.5–10 mg/kg against the P388 *in vivo* system (31) (-)-loliolide proved to be inactive.

EXPERIMENTAL⁴

ANIMAL COLLECTION.—The Western Indian Ocean (Mauritius) green sea hare *Dolabella ecaudata* was initially collected and examined in October 1972. The present study was completed with a recollection (summer 1976) of *D. ecaudata* preserved in ethyl alcohol. The total volume of sea hare preserved with ethyl alcohol amounted to approximately 44 liters.

EXTRACTION AND SOLVENT PARTITION PROCEDURES.—The ambient temperature ethyl alcohol extract from 44 liters of *D. ecaudata* was concentrated to a dark oil (1.64 kg). The total ethanol extract was partitioned between chloroform (6 x 1 liter) and water (1.5 liters); the chloroform extract was con-

⁴All solvents employed for chromatography were redistilled. Sephadex LH-20 (25–100 μ) was obtained from Pharmacia Fine Chemicals and silica gel (0.05–0.2 mm) from E. Merck (Darmstadt). Precoated Analtech Uniplates designated silica gel GHLF, were used for tlc and were developed with an anisaldehyde-acetic acid-sulfuric acid spray (34). All melting points are uncorrected and were obtained using a Koeffler melting point apparatus. Dr. J. Witschel, Jr. provided both the ¹H-nmr spectra (deuteriochloroform, TMS internal standard) using a Varian XL-100 spectrometer, and the infrared (KBr) spectra using a Beckman model 12 instrument. The ¹³C-nmr data was obtained with a Bruker WH-90 nmr spectrometer (at 22.6 MHz in ppm downfield from TMS). Mass spectra were determined with MAT CH-4B and SM-1B instruments. Optical rotations were measured using a Perkin-Elmer model 241 polarimeter.

centrated *in vacuo* to a dark green oil (10.88 g). A solution of the chloroform fraction in 9:1 methanol-water (200 ml) when extracted with hexane (9 x 100 ml) provided 3.19 g of hexane extract (32). The aqueous methanol solution was diluted to 4:1 methanol-water and extracted (9 x 100 ml) with carbon tetrachloride. Removal of the solvent from the carbon tetrachloride extract yielded a 3.26 g fraction. Final dilution of the aqueous methanol solution to 3:2 methanol-water and extraction with chloroform (7 x 100 ml) led to 3.33 g of chloroform extract.

ISOLATION OF DOLATRIOL (3).—Nearly all (3.26 g) of the chloroform fraction was preabsorbed (33) on silica gel (15 g), and the dry mixture-silica gel was added to a dry column of silica gel (225 g, 4 x 32 cm). Gradient elution chromatography, beginning with hexane and proceeding through hexane-ethyl acetate, ethyl acetate, ethyl acetate-methanol and methanol solvent systems and collecting 6 ml fractions, afforded in fractions 607–650 the diterpene dolatriol (3) previously isolated from *Dolabella auricularia* (26). The fractions containing dolatriol corresponded to 7:3 hexane-ethyl acetate. Recrystallization of dolatriol (3, 60 mg) from methylene chloride-heptane gave a pure specimen weighing 4.7 mg that was identical (infrared spectral comparison and mixture melting point determination) with an authentic specimen.

ISOLATION OF (-)-LOLIOLIDE (1).—Continued gradient elution of the silica gel chromatographic column described in the preceding experiment and collection of fractions 741–875 eluted by ethyl acetate (1.5 liters) and 99:1 ethyl acetate-methanol (500 ml) and combination of these fractions provided 0.265 g of a mixture containing (-)-loliolide (1). The total fraction (0.265 g) in 10:10:1 hexane-methylene chloride-2-propanol was carefully layered on to a column of Sephadex LH-20 (2.5 x 90 cm column) equilibrated with the same solvent mixture. Fractions of 2.5 ml each were collected, and those corresponding to 253–266 yielded 10 mg (0.0006%) of crystalline (-)-loliolide (1). Recrystallization from acetone-heptane afforded fine colorless needles melting at 153–154° (lit. ref. 12; mp 145–149° to 152–152.5° are generally found): $[\alpha]^{25D} -100^\circ$ ($C=0.07$, CHCl_3).

The (-)-loliolide isolated from *Dolabella ecaudata* was found to be identical with an authentic sample³ by infrared, and ¹³C-nmr spectral comparisons and by thin layer chromatography.

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