in mangiferin-pretreated animals, swertiamarin produced significant analgesic effect (Tables IV and V). Mangiferin significantly reduced the protective effect of swertiamarin against amphetamine (20 mg/kg ip) toxicity in aggregated mice (Table III).

The toxicity (16) and the LD_{50} of swertiamarin after single intraperitoneal administration in albino rats were studied; the LD_{50} (in milligrams per kilogram ± *SEM*) was 368 ± 45.

Earlier reports (17) indicated that iridoids are the active ingredients of some folk medicines and have been used for centuries, but the properties of specific iridoids have been evaluated only in a few cases. To the knowledge of the authors, this is the first report of a pharmacological evaluation of a pure secoiridoid, swertiamarin, occurring widely in members of the family Gentianaceae.

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ˣ To whom inquiries should be directed.

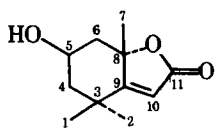
Chemical Constituents of Gentianaceae XX: Natural Occurrence of (−)-Loliolide in *Canscora decussata*

S. GHOSAL ˣ, A. K. SINGH, and R. K. CHAUDHURI *

Abstract □ (−)-Loliolide was isolated as a native compound from *Canscora decussata* Schult (Gentianaceae). Physical and spectral (UV, IR, PMR, CMR, and mass spectra) properties of the compound and its acetate derivative established its identity. The significance of the cooccurrence of loliolide with a number of carotenoids in *C. decussata* and the facile transformation of violaxanthin into loliolide and violoxin are discussed in the light of the biogenesis of the degraded carotenoid.

Keyphrases □ Loliolide—isolated from *Canscora decussata* aerial parts, transformation from violaxanthin, UV, IR, NMR, and mass spectral data □ *Canscora decussata*—loliolide isolated from extract of aerial parts, UV, IR, NMR, and mass spectral data □ Violaxanthin—transformation into loliolide and violoxin □ Carotenoids—violaxanthin, transformation into loliolide and violoxin

The isolation of nearly two dozen polyoxygenated xanthones and two tetracyclic triterpenes from different parts of *Canscora decussata* Schult (Gentianaceae) was reported previously (1, 2). This paper describes the isolation and identification of (−)-loliolide (I), as a native compound, from this species. Additionally, the significance of the cooccurrence of loliolide and a number of carotenoids (acyclic, cyclic, and xanthophylls) in *C. decussata* and the facile transformation of violaxanthin (II) into loliolide and violoxin (III) are appraised in the light of the biogenesis of the degraded carotenoid (I).



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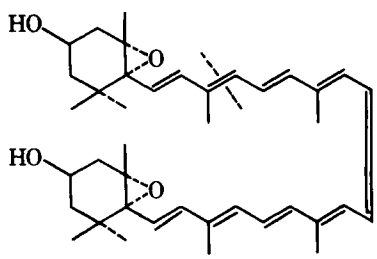

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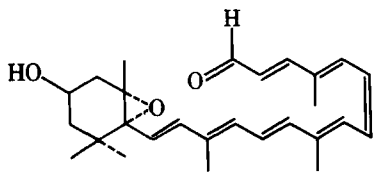


III

RESULTS AND DISCUSSION

(−)-Loliolide (I) was isolated previously (3, 4) in low yields (10^{-6}–10^{-4}%) from three plant species—*viz.*, *Lolium perenne* (Gramineae), *Digitalis purpurea*, and *D. lanata* (Scrophulariaceae). However, the process of isolation and the plant parts used did not eliminate the possibility of its formation from the carotenoids present in these plants in appreciable quantities. Thus, the question of whether loliolide is an artifact or a native compound in these plants remained unsettled. It has now been obtained in an appreciable yield (7×10^{-3}%) from aerial parts and in a lower yield (1×10^{-4}%) from roots of *C. decussata*.

The compound, mp 149–150°, $C_{11}H_{16}O_3$ (M^+, 196), showed optical rotation, UV, IR, and ^{1}H-NMR spectra identical with those reported for (−)-loliolide (3, 4). Additional spectral data (mass and ^{13}C-NMR spectra) have now been obtained for the compound and its acetate derivative. The mass spectral data can be conveniently used for the identification of loliolide even when it is obtained in a very low yield.

The mass spectrum of loliolide shows, aside from the molecular ion peak, significant fragment ion peaks at *m/e* 181 ($M - CH_3$), 178 ($M - H_2O$), 163 ($M - H_2O - CH_3$), 153 ($M - C_2H_3O$), and 111 (base peak). In the acetate derivative, only the molecular ion peak is shifted (M^+, 238), by 42 amu; the position and intensity of the fragment ion peaks remain essentially unaltered. The formation of the fragment ion species has been substantiated by metastable ions (m*).

The 100-MHz ^{1}H-NMR spectrum of loliolide (in $CDCl_3$) is very informative. Apart from the three methyl singlets appearing at 1.3, 1.5, and 1.8 ppm and a one-proton sharp singlet due to >C═CH— at 5.7 ppm, a complex pattern ranging from 1.4 to about 2.6 ppm, corresponding to five protons, is discernible. The broad resonance at 2.25 ppm is assigned to hydroxyl. The remaining signals could be analyzed by using double resonance techniques.

Irradiation of the quintuplet at 4.35 ppm ($J \sim 3.5$ Hz) showed the presence of two *AB* systems originating from magnetically nonequivalent methylene protons ($J \sim 14$ Hz). This result indicates that the molecular framework of loliolide is conformationally rigid. The small coupling ($J = 2$ Hz) is due to a four-bond long-range coupling (W-coupling). In the absence of the decoupling field, a further coupling appears (to the proton resonating at 4.35 ppm). The magnitude (3–4 Hz) for this coupling is not equal for all protons. Since the proton at 4.35 ppm is ascribable to a >CH—O function, the partial structure IV can be deduced from these data.

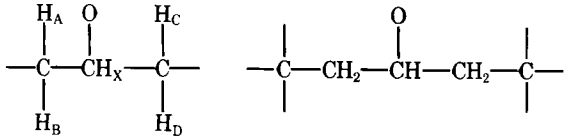
The absence of any further coupling suggests that the carbons alpha to the methylene protons are fully substituted, thus permitting extension of the partial structure IV to V. The methyl chemical shifts further imply that one quaternary carbon bears a *gem*-dimethyl group while the other one carries one methyl group and one >C—O— function.

The 25.2-MHz ^{13}C-NMR spectrum (Fourier transform) of loliolide exhibits chemical shifts and relative intensities from which straightforward assignments can be made (Table I).

The strong deshielding of the quaternary olefinic carbon (C-9) is due to its β-position relative to the lactonic carbonyl. The low intensity of the line indicates a particularly long T_1. Thus, the two other carbons (C-3 and C-8) attached to the quaternary olefinic carbon (C-9) are also quaternary. The two lines around 26 ppm indicate that one of these two quaternary carbons bears a *gem*-dimethyl group; the line around 30 ppm indicates that the other one bears a methyl and an oxygen function.

The line at 183 ppm is due to an α,β-unsaturated carbonyl. The high intensity of the line at 112 ppm must be due to a proton-bearing olefinic carbon (C-10). The remaining lines at 45, 47, and 66 ppm are due to methylene (C-4 and C-6) and hydroxymethine (C-5) carbons, respectively. The spectral data thus fit in excellently with Structure I for the C_{11} compound isolated from *C. decussata*.

Several naturally occurring compounds of terpene origin are known to arise through oxidative degradation in terpene biosynthesis. The



IV V

Table I—^{13}C-NMR Spectral Data of Loliolide

Carbon	δ_C, ppm (Relative to Internal Tetramethylsilane)
1	26.45
2	26.95
3	35.98
4	45.29
5	66.42
6	47.22
7	30.62
8	87.08
9	172.10
10	112.54
11	183.03

compounds have such degraded structures that it becomes difficult to postulate their precise biosynthetic precursors. Thus, the ionones (C_{13} terpenes) may be built up of monoterpenes (C_{10} unit), degraded sesquiterpenes (C_{15} unit), or degraded carotenoids (C_{40} unit), while the carotenoid origin is currently favored (5, 6). Isoe *et al.* showed that photooxygenation of β-carotene yields dihydroactinidiolide, β-ionone, and 2-hydroxy-2,6,6-trimethylcyclohexanone (5) and that exhaustive photooxygenation of zeaxanthin yields (−)-loliolide and (+)-isololiolide plus other products (6). Taylor and Burden (7) reported that violaxanthin (II), on photochemical oxidation, yields loliolide, 3-hydroxy-5,6-epoxy-β-ionone, and xanthoxin. Lutein *trans*-epoxide was shown recently (8) to produce loliolide on cautious oxidation with nickel peroxide.

The weight of evidence thus favors that loliolide is a degraded carotenoid, and evidence from the present study supports this conclusion. From a benzene solution of II, loliolide and a C_{27} aldehyde (named violoxin) were isolated as products of autoxidation. Violoxin, an oil, $C_{27}H_{36}O_3$ (M^+, 408), shows an orange spot by TLC when sprayed with 2,4-dinitrophenylhydrazine. Benzene solution of violoxin on prolonged sitting also furnishes loliolide. These results and the spectral properties are consistent with Structure III for violoxin. During autoxidation, II seems to be cleaved at the dotted line, yielding 3-hydroxy-5,6-epoxy-β-ionone (or equivalent) and violoxin (III). The formation of loliolide from 3-hydroxy-5,6-epoxy-β-ionone is a well-documented phenomenon (6).

Definite evidence has now been obtained that loliolide is a native compound in *C. decussata*. A qualitative survey of the distribution of carotenoids in flowers and fruits of some members of the genera *Canscora*, *Gentiana*, and *Swertia*, belonging to the family Gentianaceae, was made recently by chromatographic and spectroscopic methods (9, 10). From aerial parts of *C. decussata*, five carotenoids—*viz.*, phytoene, phytofluene, β-carotene, lutein epoxide, and violaxanthin, were isolated and identified. The roots of the plant, however, were found to be free from carotenoids.

The isolation of loliolide from the roots, therefore, suggests that it is a native compound in *C. decussata* and is not formed by the autoxidation of any one of the oxycarotenoids during the extraction of these compounds. A portion of loliolide probably is formed as a metabolite of one of the oxycarotenoids (or equivalent) *in vivo* moves to the roots from the flowers and fruits of *C. decussata* during the growth of the plant. The possible role of this and related carotenoid metabolites in the ontogeny of plants is currently being investigated.

EXPERIMENTAL[1]

Extraction of *C. decussata*[2]—In a typical experiment, air-dried and milled aerial parts (2.1 kg) of the plant, collected from the local area and properly identified[3], were extracted (30 hr) under reflux in a 3-liter soxhlet apparatus with petroleum ether (bp 60–80°). The petroleum ether extract was concentrated (to about 400 ml), and the concentrate was extracted with aqueous citric acid (12.5%, 200 ml) using a mechanical stirrer (8 hr).

[1] The general directions are the same as were reported recently (1).

[2] The plant material was supplied by Mr. B. Singh. A voucher specimen has been preserved in the Pharmaceutical Chemistry Research Laboratory, Department of Pharmaceutics, Banaras Hindu University.

[3] Through the courtesy of Dr. C. S. P. Rao, Department of Botany, Banaras Hindu University.

Isolation of (−)-Loliolide (I)—The clarified aqueous citric acid solution was cooled and made basic with ammonia (pH 8.5–9.0), and this solution was extracted with chloroform (five 50-ml portions). The chloroform extract was washed and dried, and the solvent was removed; a straw-colored amorphous solid (0.51 g) was obtained. It was again dissolved in chloroform (10 ml) and chromatographed over alumina (30 g). The elution was carried out with benzene. Fractions (50 ml) were collected and monitored by analytical TLC. *n*-Butyl alcohol–acetic acid–water (4:1:2) was used as the solvent system, and iodine vapor was used for staining.

Fractions 9–11 were combined and evaporated. The residue crystallized from acetone–petroleum ether (1:2) as colorless fine needles (143 mg), mp 149–150° [lit. (3, 4) mp 149°, 149–151°]; R_f 0.82; $[\alpha]_D^{25}$ −116.3° (c 0.98, $CHCl_3$); UV: λ_{max} (ethanol) 216 nm (log ϵ, 4.17); IR: ν_{max} (mineral oil) 3425, 1720, and 1622 cm^{-1}; mass spectrometry: *m/e* 196 (M^+, 36%), 181 (7), 179 (5), 178 (50, m* 162, $178^2/196 = 161.6$), 163 (15, m* 149.5, $163^2/178 = 149.2$), 153 (16), 140 (50), 135 (15), 112 (18), and 111 (100).

Anal.—Calc. for $C_{11}H_{16}O_3$: C, 67.34; H, 8.16. Found: C, 67.32; H, 8.16.

The acetyl derivative, prepared with pyridine and acetic anhydride (in equal proportions) at ordinary temperature for 40 hr, was purified by column chromatography over alumina[4]. Benzene–ether (1:1) was used as the eluent. The middle eluates furnished loliolide acetate, which crystallized from hexane–methylene chloride as colorless prisms, mp 86–87° [lit. (3, 4) mp 86–87°, 86.5°]; $[\alpha]_D^{25}$ −74.7° (c 0.77, $CHCl_3$); UV: λ_{max} (ethanol) 214 nm (log ϵ, 4.28); mass spectrometry: *m/e* 238 (M^+, 21%), 223 (4), 195 (12), 178 (54), 163 (11), 135 (8), and 111 (100).

Autoxidation of Violaxanthin (II)—Violaxanthin (52 mg), obtained from *C. decussata* (10), was dissolved in benzene (50 ml) containing traces of methanol and was kept at room temperature for about 3 weeks. The yellow color of the solution faded gradually, and the solution was practically colorless at the end of the reaction. After evaporation of the solvent, the residue was partitioned between aqueous methanol and petroleum ether (bp 60–80°). The residue from the methanol layer was dissolved in benzene and chromatographed over a silicic acid (100-mesh) column, using benzene–ethyl acetate (2:1) as the eluent.

Fractions (50 ml) were collected, and fractions 10–15 showed the presence of loliolide and an aldehyde (2,4-dinitrophenylhydrazine reagent positive) component. The latter had a slightly lower R_f, 0.62, in acetone–petroleum ether (1:3). These compounds were separated by preparative TLC (plate thickness, 2 mm) with the same solvent system. The component having the lower R_f value was identified as violoxin.

Violoxin (III)—This compound was obtained as an oil (11 mg); UV: λ_{max} (ethanol) 230 (0.56), 262 sh (0.38), and 300 (0.08) nm; IR: ν_{max} (liquid) 3450, 1658, 1628, and 972 cm^{-1}; mass spectrometry: *m/e* 408 (51%), 393 (26), 390 (5), and 375 (12).

[4] Brockman alumina, grade IV.

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* Present address: Pharmazeutisches Institut der Universität, Bonn, West Germany.

[x] To whom inquiries should be directed.

Antimicrobial Activity of Newly Synthesized Isothiocyanate Derivatives against Pathogenic Plant Microorganisms

MISAO KOJIMA [x] and M. KADO *

Abstract □ Fifteen reaction products of isothiocyanates with cysteine, seven reaction products of isothiocyanates with 2,3-dimercapto-1-propanol, and four reaction products of isothiocyanates with sulfanilamide were synthesized. Their antimicrobial activity against pathogenic plant microorganisms was investigated.

Keyphrases □ Isothiocyanate derivatives—synthesized and screened for antimicrobial activity □ 1-Propanols, 2,3-bis(alkylthiocarbamoylthio)—synthesized and screened for antimicrobial activity □ Cysteines, *N*-(*N*-substituted thiocarbamoyl)—synthesized and screened for antimicrobial activity □ Thioureas, *N*-substituted *N'*-*p*-sulfonamidophenyl—synthesized and screened for antimicrobial activity □ Antimicrobial activity—isothiocyanate derivatives screened □ Structure–activity relationships—isothiocyanate derivatives synthesized, screened for antimicrobial activity

It was reported previously (1) that some reaction products of isothiocyanates with cysteine and 2,3-dimercapto-1-propanol (dimercaprol) exhibited antimicrobial activity against some bacteria and fungi. This paper reports the antimicrobial activity against pathogenic plant microorganisms of these compounds and