(11) M. Katayama, S. Fujiwara, H. Suzuki, Y. Nagai, and O. Simamura, *J. Mol. Spectrosc.*, **5**, 85(1960).

ACKNOWLEDGMENTS AND ADDRESSES

Received October 20, 1975, from *Merrell-National Laboratories, Division of Richardson-Merrell Inc., Cincinnati, OH 45215, and the ‡Section of Medicinal Chemistry and Pharmacognosy, School of Pharmacy, University of Connecticut, Storrs, CT 06268

Accepted for publication December 17, 1975.

Supported in part by the Department of Health, Education, and Welfare, U.S. Public Health Service, under special Postdoctoral Fellowship GM 49037.

x To whom inquiries should be directed.

Chemical Constituents of Gentianaceae XIX: CNS-Depressant Effects of Swertiamarin

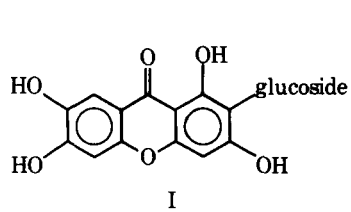
S. K. BHATTACHARYA *, P. K. S. P. REDDY *, S. GHOSAL‡x, A. K. SINGH ‡, and P. V. SHARMA ‡

Abstract □ CNS activity of swertiamarin, a secoiridoid glucoside from *Swertia chirata*, was evaluated. An apparent anomaly, associated with the unanticipated finding that the alcoholic extracts (excluding mangiferin) of *S. chirata* significantly reversed the mangiferin-induced CNS-stimulating effects in albino mice and rats, was resolved. The results indicate that swertiamarin and mangiferin antagonize each other *in vivo* and thereby reverse their CNS effects.

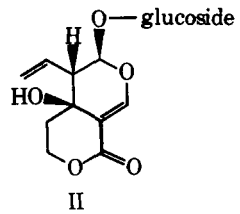
Keyphrases □ Gentianaceae—*Swertia chirata*, CNS activity of swertiamarin evaluated □ Swertiamarin—CNS activity evaluated, interaction with mangiferin □ CNS activity—swertiamarin evaluated □ Mangiferin—CNS activity, effect of swertiamarin

One result of a prior study, dealing with the active principles of *Swertia chirata* (Gentianaceae) (1), was the unanticipated finding that the alcoholic extracts, containing xanthones (excluding mangiferin) and secoiridoid glucosides, in doses of 50–100 mg/kg ip, significantly reversed the mangiferin-induced hyperactivity in albino mice and rats and also the potentiating effect of mangiferin on amphetamine toxicity in aggregated mice. Mangiferin (I), isolated in appreciable quantities from *S. chirata* (1.2 g/kg of whole plant) (1) and from *Canscora decussata* (30 g/kg of whole plant) (2), was earlier shown to produce significant pharmacological actions on the central nervous system (CNS) of laboratory animals (3, 4). In doses of 50–100 mg/kg ip, it produced definite signs of CNS stimulation in albino mice and rats as evidenced by hyperactivity, fine tremors, piloerection, increased spontaneous motility, reversal of reserpine-induced ptosis and sedation, potentiation of the analgesic effect of subanalgesic doses of morphine, and potentiation of amphetamine toxicity in aggregated mice.

The actions of mangiferin were subsequently found to be mediated *via* monoamine oxidase inhibition (5). Also, it was shown recently (6) that mangiferin-induced potentiation of the antinociceptive effect of morphine, like that of nialamide, was 5-hydroxytryptamine mediated. This paper describes findings that indicate that the reversal of mangiferin-induced pharmacological effects by the alcoholic extracts of *S. chirata* is due to the presence of an appreciable quantity (about 4 g/kg of whole plant) (5) of a CNS depressant swertiamarin (II).



I



II

EXPERIMENTAL

The isolation and purification of swertiamarin from *S. chirata* were reported previously (5). Pharmacological studies were conducted on albino mice (18–25 g) and albino rats[1] (80–120 g). The animals were fed a standard pellet diet[2]. All experiments were conducted at ambient temperature of 24 ± 2°.

The drug was tested in doses of 25–100 mg/kg ip. Unless stated otherwise, the data given indicate the effect of swertiamarin in doses of 50 mg/kg ip and a pretreatment time of 1 hr. In experiments where combined effects of mangiferin and swertiamarin were investigated, mangiferin was administered at 100 mg/kg ip and the pretreatment time was 2 hr.

In all experiments, 10 animals were used for the drug-treated and control groups; controls received only the vehicle, distilled water. Statistical analyses were done by the Student t test and χ-square test at appropriate places.

RESULTS AND DISCUSSION

In primary observational tests (7), swertiamarin produced a transient stimulation followed by signs of marked central depression in albino mice. The initial stimulation was absent in albino rats. The depressant activity was characterized by considerably diminished spontaneous motility, grouping of animals on one side of the cage, and ptosis. However, reflexes were intact, and the animals responded to external stimuli.

With higher doses (75–100 mg/kg ip), normal activities of the mice such as grooming were further reduced, the gait became abnormal, and the animals lost their ability to remain on an inclined plane. They responded to painful stimuli, although the righting reflex was sluggish. In these doses, swertiamarin produced significant hypothermia in albino rats as recorded by a rectal thermister probe (Table I). Since these observations were indicative of CNS depressant activity, swertiamarin was subjected to further pharmacological screening as follows.

The effects of swertiamarin on hexobarbital hypnosis (8) and amphetamine toxicity in aggregated and isolated mice (9) were evaluated

[1] The animals were supplied by Messrs B. N. Ghosh & Co., Calcutta, India.

[2] Hindusthan Levers, Calcutta, India.

Table I—Effect of Swertiamarin on Rectal Temperature (RT), Spontaneous Motor Activity (SMA), Forced Locomotor Activity (FLA) (Rotarod Test), and Pentylenetetrazol-Induced Convulsions (PIC)[a]

Group	Dose, mg/kg ip	RT, Mean ± *SEM*	SMA, Mean ± *SEM*	FLA, % Fall within 180 sec	PIC, % Incidence of Convulsions
Control	—	36.5 ± 0.96°	298 ± 42	0	100
Swertiamarin	50	35.2 ± 0.21°, $p > 0.05$[b]	74 ± 14, $p < 0.01$	70, $p < 0.01$	50, $p > 0.05$
Swertiamarin	100	34.3 ± 0.34°, $p < 0.05$	33 ± 9, $p < 0.001$	90, $p < 0.001$	70, $p < 0.01$

[a] Number of animals was 10 in each case. [b] The p value shows significance in relation to respective control groups.

(Tables II and III). Swertiamarin significantly potentiated (110% over the control) hexobarbital- (100 mg/kg ip) induced sleeping time in albino mice. It diminished amphetamine- (20 mg/kg ip) induced toxicity in both aggregated and isolated mice in similar doses. The ED_{50}'s of swertiamarin (in milligrams per kilogram ± *SEM*) against the lethal effect of amphetamine in aggregated and isolated mice were 6.25 ± 5.9 and 58.6 ± 6.3, respectively.

The effects of swertiamarin on rats and mice on the rotarod test (10) and on their conditioned avoidance response (11) were examined. It markedly inhibited the ability of trained mice to remain on a rotating rod for a maximum trial of 180 sec. With higher doses, the incidence of fall increased (Table I). Swertiamarin had no effect on the avoidance response to the conditioned stimulus (buzzer) in trained rats. However, in higher doses (75–100 mg/kg ip), it significantly blocked the escape response to both conditioned and unconditioned (electric shock) stimuli in 65% of the animals ($p < 0.05$) and there was a marked loss of motor tone.

Table II—Effect of Swertiamarin on Hexobarbital Hypnosis in Absence and Presence of Mangiferin[a]

Group	Drugs (Dose, mg/kg ip)	Hexobarbital Sleeping Time, min, Mean ± *SEM*	p
1	Hexobarbital (100)	21.4 ± 1.4	
2	Swertiamarin (50) plus hexobarbital (100)	43.8 ± 4.3	<0.001[b]
3	Mangiferin (100) plus hexobarbital (100)	31.8 ± 3.2	<0.01[b]
4	Mangiferin (100) plus swertiamarin (50) plus hexobarbital (100)	56.3 ± 3.1	<0.01[c]

[a] Number of animals was 10 in each case. [b] Significance in relation to Group 1. [c] Significance in relation to Group 2.

Table III—Effect of Swertiamarin on Amphetamine Toxicity in Aggregated Mice in Absence and Presence of Mangiferin[a]

Group	Drugs (Dose, mg/kg ip)	Mortality (18 hr), %	p
1	Amphetamine (20)	100	—
2	Swertiamarin (50) plus amphetamine (20)	60	>0.05[b]
3	Swertiamarin (100) plus amphetamine (20)	20	<0.001[b]
4	Mangiferin (100) plus amphetamine (20)	100	—
5	Mangiferin (100) plus swertiamarin (100) plus amphetamine (20)	80	<0.05[c]
6	Amphetamine (10)	20	—
7	Mangiferin (100) plus amphetamine (10)	80	<0.05[d]
8	Mangiferin (100) plus swertiamarin (100) plus amphetamine (10)	40	>0.05[e]

[a] Number of animals was 10 in each case. [b] Significance in relation to Group 1. [c] Significance in relation to Group 3. [d] Significance in relation to Group 6. [e] Significance in relation to Group 7.

The effects of swertiamarin on morphine analgesia (12), anticonvulsant action of phenytoin (13), electroshock seizure (13), and pentylenetetrazol convulsion (14) were also determined (Tables I, IV, and V). Swertiamarin significantly potentiated the analgesic activity of a subanalgesic dose (2 mg/kg ip) of morphine (15) but had no analgesic activity *per se*. It also significantly potentiated the anticonvulsant activity of a subanticonvulsant dose (2.5 mg/kg ip) of phenytoin but had no anticonvulsant activity *per se*. In higher doses (75–100 mg/kg ip), swertiamarin offered significant protection against pentylenetetrazol- (70 mg/kg sc) induced convulsion. However, as mentioned earlier, there was marked loss of motor tone in the animals with these doses.

In view of the initial observation and the cooccurrence of mangiferin and swertiamarin as the two major chemical constituents of *S. chirata*, their interactions on selected pharmacological parameters were examined. Mangiferin significantly inhibited swertiamarin-induced sedation, ptosis, and hypothermia. It further potentiated (160%) swertiamarin-induced potentiation (110%) of hexobarbital-induced sleeping time in mice (Table II). Since mangiferin itself potentiated (50%) hexobarbital hypnosis in mice, the combined effect appears to be additive.

In mangiferin-pretreated animals, swertiamarin produced significant anticonvulsant effect (against electroshock seizure). Likewise,

Table IV—Effect of Swertiamarin on Morphine Analgesia and Its Analgesic Effect *per se* in Absence and Presence of Mangiferin[a]

Group	Drugs (Dose, mg/kg ip)	Latent Period of Tail-Flick Response, sec, Mean ± *SEM*	p
1	Morphine (2)	3.9 ± 0.7	—
2	Swertiamarin (50)	2.4 ± 0.96	—
3	Swertiamarin (50) plus morphine (2)	10.7 ± 1.1	<0.001[b]
4	Mangiferin (100)	3.2 ± 0.7	—
5	Mangiferin (100) plus swertiamarin (50)	9.3 ± 1.06	<0.001[c]

[a] Number of animals was 10 in each case. [b] Significance in relation to Group 1. [c] Significance in relation to Group 2.

Table V—Effect of Swertiamarin on Anticonvulsant Effect of Phenytoin and Its Anticonvulsant Effect *per se* against Electroshock Seizure in Absence and Presence of Mangiferin[a]

Group	Drugs (Dose, mg/kg ip)	Anticonvulsant Effect, %	p
1	Phenytoin (2.5)	0	—
2	Swertiamarin (50)	0	—
3	Swertiamarin (100)	40	>0.05[b]
4	Swertiamarin (50) plus phenytoin (2.5)	60	<0.05[b]
5	Mangiferin (100)	0	—
6	Mangiferin (100) plus swertiamarin (50)	60	<0.05[c]

[a] Number of animals was 10 in each case. [b] Significance in relation to Group 1. [c] Significance in relation to Group 2.

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.

REFERENCES

(1) S. Ghosal, P. V. Sharma, R. K. Chaudhuri, and S. K. Bhattacharya, *J. Pharm. Sci.*, **62**, 926(1973).

(2) R. K. Chaudhuri and S. Ghosal, *Phytochemistry*, **10**, 2425(1971).

(3) S. K. Bhattacharya, S. Ghosal, R. K. Chaudhuri, and A. K. Sanyal, *J. Pharm. Sci.*, **61**, 1838(1972).

(4) S. K. Bhattacharya, A. K. Sanyal, and S. Ghosal, *Naturwissenschaften*, **59**, 650(1972).

(5) P. V. Sharma, Ph.D. thesis, Banaras Hindu University, Varanasi, India, 1974, p. 118.

(6) S. K. Bhattacharya, A. K. Sanyal, and S. Ghosal, in "Drugs and Central Synaptic Transmission," P. B. Bradley and B. N. Dhawan, Eds., Macmillan, London, England, 1975, p. 94.

(7) R. A. Turner, in "Screening Methods in Pharmacology," vol. 1, Academic, New York, N.Y., 1965, p. 26.

(8) W. L. Kuhn and E. F. Van Maanen, *J. Pharmacol. Exp. Ther.*, **134**, 60(1961).

(9) J.H. Mennear and A. D. Rudzik, *Life Sci.*, **5**, 349(1966).

(10) W. J. Kinnard and C. J. Carr, *J. Pharmacol. Exp. Ther.*, **121**, 354(1957).

(11) L. Cook and E. Weidley, *Ann. N.Y. Acad. Sci.*, **66**, 740(1957).

(12) O. L. Davies, J. Faventos, and W. J. Walpole, *Br. J. Pharmacol.*, **1**, 255(1946).

(13) E. A. Swinyard, W. C. Brown, and W. K. Young, *J. Pharmacol. Exp. Ther.*, **106**, 319(1952).

(14) L. S. Goodman, M. S. Grewal, W. C. Brown, and E. A. Swinyard, *ibid.*, **108**, 168(1953).

(15) S. K. Bhattacharya, M. K. Raina, D. Banerjee, and N. C. Neogi, *Indian J. Exp. Biol.*, **9**, 257(1971).

(16) L. C. Miller and M. L. Tainter, *Proc. Soc. Exp. Biol. Med.*, **57**, 261(1944).

(17) J. M. Bobbit and K.-P. Segebarth, in "Cyclopentanoid Terpene Derivatives," W. I. Taylor and A. R. Battersby, Eds., Dekker, New York, N.Y., 1969, p. 135.

ACKNOWLEDGMENTS AND ADDRESSES

Received April 7, 1975, from the *Departments of *Pharmacology and ‡Pharmaceutics, Banaras Hindu University, Varanasi-5, India.*

Accepted for publication January 6, 1976.

The authors thank the Council of Scientific and Industrial Research, New Delhi, India, for financial assistance.

ˣ To whom inquiries should be directed.

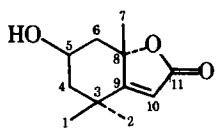
Chemical Constituents of Gentianaceae XX: Natural Occurrence of (−)-Loliolide in *Canscora decussata*

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

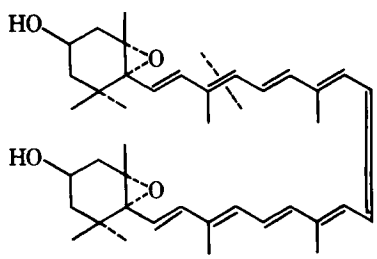
Abstract □ (−)-Loliolide was isolated as a native compound from *Canscora decussata* Schult (Gentianaceae). Physical and spectral (UV, IR, PMR, CMR, and mass spectra) properties of the compound and its acetate derivative established its identity. The significance of the cooccurrence of loliolide with 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

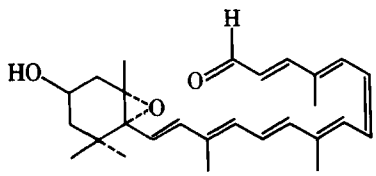
The isolation of nearly two dozen polyoxygenated xanthones and two tetracyclic triterpenes from different parts of *Canscora decussata* Schult (Gentianaceae) was reported previously (1, 2). This paper describes the isolation and identification of (−)-loliolide (I), as a native compound, from this species. 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).



I



II



III