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# Chemical Constituents of the Gentianaceae V: Tetraoxygenated Xanthenes of *Swertia chirata* Buch.-Ham.

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**Abstract** □ Nine tetraoxygenated xanthenes [1,5,8-trihydroxy-3-methoxyxanthone (I), 1-hydroxy-3,5,8-trimethoxyxanthone (II), 1-hydroxy-3,7,8-trimethoxyxanthone (III), 1,8-dihydroxy-3,5-dimethoxyxanthone (IV), 1,8-dihydroxy-3,7-dimethoxyxanthone (V), 1,3,6,7-tetrahydroxyxanthone- $\beta$ -D-glucoside (mangiferin, VI), 1,3,8-trihydroxy-5-methoxyxanthone (VII), 1,3,5,8-tetrahydroxyxanthone (VIII), and 1,3,7,8-tetrahydroxyxanthone (IX)] were isolated from the roots and aerial parts of *Swertia chirata* Buch.-Ham. (Gentianaceae) collected from Nepal and India. The identity of the xanthenes was established by direct comparison with reference materials in most cases, preparation of derivatives, and spectral evidence (UV, IR, proton magnetic resonance, and mass spectrometry). Among these xanthenes, II was not encountered before in nature and VI was found for the first time in the genus *Swertia*. The biogenetic significance of the co-occurrence of several tetraoxygenated xanthenes, of varying oxygenation patterns, in absence of the "standard" 1,3,5- and 1,3,7-trioxygenated xanthenes in *S. chirata* and in related species is appraised. In addition to the tetraoxygenated xanthenes (I-IX), a number of heterosides, triterpenes, and monoterpene alkaloids were isolated from this plant. Preliminary pharmacological screening of the total xanthenes of *S. chirata* indicated that the medicinal properties ascribed to the plant extracts were due to these constituents.

**Keyphrases** □ *Swertia chirata* Buch.-Ham. (Gentianaceae)—isolation, identification of nine tetraoxygenated xanthenes □ Xanthenes, tetraoxygenated— isolation, identification from *Swertia chirata* Buch.-Ham. (Gentianaceae) □ Medicinal plants— isolation, identification of tetraoxygenated xanthenes from *Swertia chirata* Buch.-Ham.

*Swertia chirata* Buch.-Ham. (Gentianaceae) is widely distributed in India in the temperate Himalayas between 4000 and 10,000 ft., from Kashmir to Bhutan, and in Khasia Hills between 4000 and 5000 ft. (1). It also grows abundantly in Nepal. The plant is well known for its uses in the Indian system of medicine for a variety of purposes (1, 2). The extract of the plant is used as a bitter stomachic, a febrifuge, an anthelmintic, a remedy for scanty urine, in epilepsy, and for certain types of mental disorders. The total annual requirement of this pharmacopeial drug in India is about 400 quintals (3).

Previous phytochemical investigation by Dalal and Shah (4) reported the presence of only one xanthone, 1,8-dihydroxy-3,5-dimethoxyxanthone, in the whole plant. But no attempt was made to determine the active

principle of this vegetable drug. Interest in the detailed investigation of this plant was piqued for two reasons: (a) xanthone-bearing plants generally elaborate multiple xanthenes (5), and (b) in *Calophyllum inophyllum* L. (Guttiferae), variations in the types of xanthonic constituents were recorded (6-8) due to ecological variations. In the present study, *S. chirata* plants<sup>1</sup> were collected from Nepal and India to test these possibilities.

## EXPERIMENTAL<sup>2</sup>

The general procedure described under *Isolation of Xanthenes from Aerial Parts of S. chirata* was followed for the isolation of xanthenes from the roots. The other chemical constituents were isolated following a procedure shown in Scheme I.

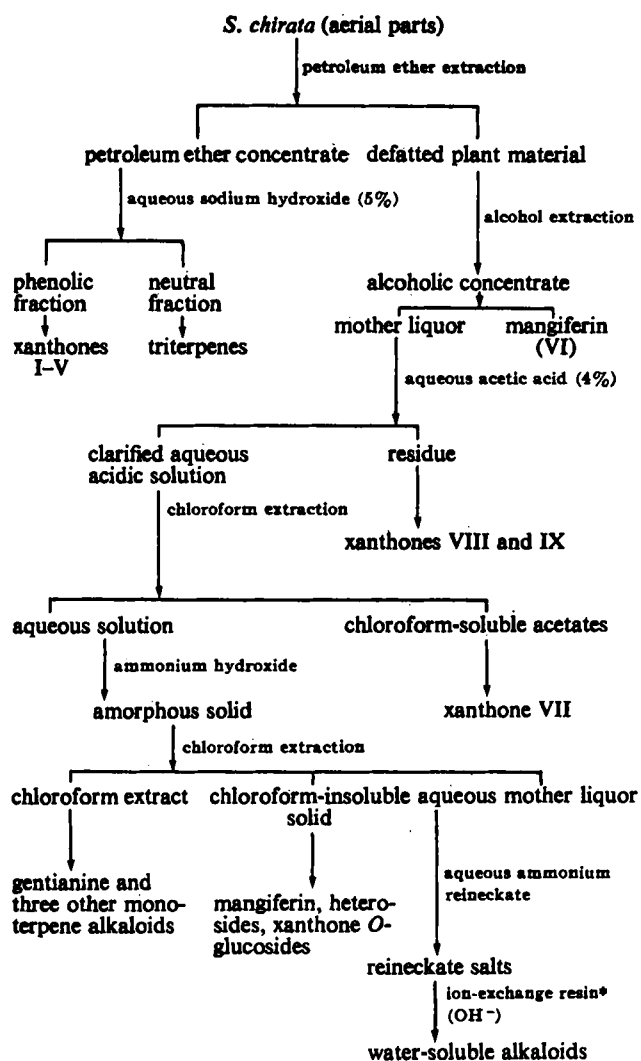
**Isolation of Xanthenes from Aerial Parts of *S. chirata***—Dried and milled aerial parts of *S. chirata* (1 kg.) were continuously extracted (soxhlet) for 20 hr. with petroleum ether (60-80°), and the defatted plant material was subsequently extracted (20 hr.) with ethanol. The two extracts were separately processed.

**Treatment of Petroleum Ether Extract**—The petroleum ether extract was concentrated (about 250 ml.) under reduced pressure, and the concentrate was kept overnight at ordinary temperature when a yellow amorphous solid (Fraction A) separated. The solid was collected by filtration, and the mother liquor was evaporated to dryness (Fraction B).

**Separation of Xanthenes Present in Fraction A**—Fraction A (1.8 g.) was dissolved in ether (500 ml.), and the phenolic and non-phenolic components were separated by extraction with aqueous

<sup>1</sup> The plant material of Indian origin was a gift from Dr. S. P. Wahi, Department of Pharmaceutics, Banaras Hindu University, and that from Nepal was supplied by Mr. R. A. Panjiar, Janakpur Dhum, Nepal. Voucher specimens have been preserved at the Department of Pharmaceutics, Banaras Hindu University, Varanasi-5, India.

<sup>2</sup> Melting points were determined on a Toshniwal melting-point apparatus, in open capillaries, and are uncorrected. UV spectra were recorded in a Cary 14 spectrophotometer in aldehyde-free ethanol (95%). IR spectra were recorded with Perkin-Elmer 237/257 instruments in KBr and mineral oil. Proton magnetic resonance (PMR) spectra were obtained on a Varian A-60 D spectrometer, using deuteriochloroform and dimethyl sulfoxide- $d_6$  as the solvents. Mass spectra were determined on an AEI MS-9 instrument operated at 70 eV. Combustion analyses were performed by the Central Drug Research Institute, Lucknow, India. Separation by column chromatography was carried out by using silica gel (60-120 mesh, British Drug Houses), and layer chromatographic experiments were conducted with silica gel G (E. Merck). Four solvent systems [benzene-acetic acid (60:1, Solvent 1), chloroform-benzene (5:2, Solvent 2), chloroform-benzene (1:1, Solvent 3), and *n*-butyl alcohol-acetic acid-water (4:1:2, Solvent 4)] were used as the developers, and iodine vapor was used for staining purposes.



Scheme 1—Isolation of chemical constituents of *S. chirata* (\*Amberlite IRA 400)

sodium hydroxide (5%, four 25-ml. portions). The phenolic constituents from the alkaline aqueous extracts were liberated with hydrochloric acid (2 *N*) and then extracted with chloroform (three 50-ml. portions). The neutral fraction, obtained from the ethereal layer, consisted mainly of triterpenes and was kept aside. The phenolic constituents (118 mg.) showed one major spot and one minor spot on analytical thin-layer chromatoplates. The major component was purified by column chromatography (silica gel, 100 g.), using petroleum ether and benzene as the eluents. The petroleum ether eluates did not give any solid, and the small amount of the gummy material obtained was not processed further.

**Xanthone I (1,5,8-Trihydroxy-3-methoxyxanthone)**—The benzene eluates, on concentration, gave a yellow solid (52 mg.), which crystallized from ethanol as yellow shining needles, m.p. 271° [lit. (9) m.p. 270–271°];  $R_f$  0.5 (Solvent 2);  $\nu_{\max}$  (KBr): 3400, 1668, 1628, 1610, 1045, and 995  $\text{cm}^{-1}$ .

The 5,8-dimethyl ether, prepared with ethereal diazomethane, crystallized from alcohol as pale-yellow needles, m.p. and mixed m.p. 205°.

**Separation of Xanthenes Present in Fraction B**—Fraction B (0.8 g.) was dissolved in ether (300 ml.), and the phenolic and non-phenolic constituents were separated as described for Fraction A. The phenolic constituents (248 mg.), obtained as a brown gum, were dissolved in chloroform (15 ml.) and chromatographed over silica gel (250 g.). Elution was carried out with petroleum ether (2.5 l.) and benzene (4 l.). The eluates, on evaporation, gave amorphous solids.

The solid (132 mg.), obtained from the petroleum ether eluates, showed several spots on analytical thin-layer chromatoplates. It was dissolved in benzene (10 ml.) and again chromatographed over

silica gel (150 g.). The middle petroleum ether–benzene (1:9) eluates showed two major spots on analytical thin-layer plates. These were separated by preparative layer chromatography, using Solvent 3 as the developer.

**Xanthone II (1-Hydroxy-3,5,8-trimethoxyxanthone)**—The yellow upper zone from the preparative layer chromatography was scraped and eluted with chloroform. Evaporation of the solvent gave a solid (61 mg.), which crystallized from ethanol as pale-yellow needles, m.p. and mixed m.p. with an authentic synthetic sample (12) 205°;  $\nu_{\max}$  (KBr): 3428 (broad), 2912, 2858, 1662, 1615, 1598, and 1060  $\text{cm}^{-1}$ .

**Xanthone III (1-Hydroxy-3,7,8-trimethoxyxanthone)**—The deep-yellow lower layer, from the preparative layer chromatography, gave yellow needles from ethanol, m.p. and mixed m.p. with authentic decussatin (10) 148–149°. The IR spectra were also superimposable.

The solid (124 mg.), obtained from the benzene eluates (Fraction B), showed several spots on analytical thin-layer chromatoplates. It was dissolved in chloroform (10 ml.) and rechromatographed on silica gel (150 g.). Petroleum ether, benzene, and different proportions of their mixtures were used as the eluents. The petroleum ether–benzene (1:1) fraction showed two major spots on TLC, which were separated by preparative layer chromatography using Solvent 3 as the developer.

**Xanthone IV (1,8-Dihydroxy-3,5-dimethoxyxanthone)**—The yellow upper zone from the layer chromatography afforded a solid (48 mg.), which crystallized from ethanol as yellow needles, m.p. 185–186° [lit. (9) m.p. 185–186°];  $R_f$  0.68 (Solvent 3);  $\nu_{\max}$  (KBr): 3438, 2925, 2855, 1688, 1632, 1600, 1050, and 978  $\text{cm}^{-1}$ .

The 8-methyl ether, prepared with ethereal diazomethane, crystallized from ethanol as pale-yellow needles, m.p. and mixed m.p. with xanthone II 205°.

**Xanthone V (1,8-Dihydroxy-3,7-dimethoxyxanthone)**—The yellow lower layer from the preparative layer chromatography afforded a solid (21 mg.), which crystallized from ethanol as bright-yellow needles, m.p. 175–176°;  $R_f$  0.36 (Solvent 1); co-TLC with authentic 1,8-dihydroxy-3,7-dimethoxyxanthone (10) showed a single spot having the same  $R_f$  value. The 8-methyl ether, prepared with ethereal diazomethane, crystallized from ethanol as yellow needles, m.p. and mixed m.p. with decussatin (10) 148–149°.

**Treatment of Alcoholic Extract**—The alcoholic extract of the defatted plant material was concentrated under reduced pressure, and the concentrate was allowed to stand at ordinary temperature overnight. The precipitated dull-yellow solid (1.2 g.) was collected by filtration.

**Xanthone VI (1,3,6,7-Tetrahydroxyxanthone- $C_7\beta$ -D-glucoside)**—The above solid crystallized from dioxane as yellow needles, m.p. 270–271°. The mixed melting point with authentic mangiferin (10), m.p. 270–271°, remained undepressed. Co-TLC with mangiferin showed a single spot having the same  $R_f$  value; their IR spectra were also superimposable.

Mangiferin heptaacetate, prepared with boiling acetic anhydride–pyridine, crystallized from chloroform–hexane (1:4) as colorless needles, m.p. 214–216° [lit. (11) m.p. 228–230°, 141°]. The heptaacetate was also prepared with acetic anhydride and sulfuric acid (1 drop) at ordinary temperature. The derivative crystallized from chloroform–hexane as colorless microcrystals, m.p. 212–214°. The mixed melting point of the heptaacetates, prepared under basic and acidic conditions, remained undepressed. The heptaacetate did not give any color with ferric chloride. The IR, PMR, and mass spectral data of the compound were also consistent with the assigned structure.

The alcoholic mother liquor, after separation of mangiferin, was concentrated to a syrupy liquid. It was poured into aqueous acetic acid (4%, 400 ml.). The mixture was kept at ordinary temperature overnight when an amorphous solid separated. It was filtered and the solid was extracted with chloroform. The chloroform-insoluble solid was processed for polyhydroxylated xanthenes (Fraction C). The chloroform-soluble fraction was combined with the chloroform-soluble acetates. The clarified aqueous acidic solution was extracted with chloroform (four 100-ml. portions) to remove the chloroform-soluble acetates. The combined chloroform extracts were washed with water, dried (anhydrous sodium sulfate), and evaporated to give a yellow solid (82 mg.).

**Xanthone VII (1,3,8-Trihydroxy-5-methoxyxanthone)**—The above solid was triturated with ethanol when a further crop (18 mg.) of mangiferin (VI) was obtained from the alcohol-insoluble frac-

Table I—UV Spectral Data of the Xanthoness<sup>a</sup> of *S. chirata*

Xanthone	$\lambda_{\text{max}}^{\text{EtOH}}$ , nm. (log $\epsilon$ )
I	220 (4.28), 240 (4.15), 255 (4.20), 277 (4.10), 305–310 sh (3.71), 329 (3.92)
II	220 (4.08), 230–235 sh (4.15), 250 (4.40), 274 (3.92), 332 (3.88)
IV	235 (4.30), 251 (4.42), 275 (4.21), 295–297 (3.80), 332 (3.98)
VII	220 (4.20), 252 (4.32), 276 (4.18), 305–310 sh (3.73), 339 (4.08)
VIII	239 (4.28), 267 (4.52), 332 (4.10), 390 (3.88)

<sup>a</sup> UV data of other xanthoness were previously recorded (10).

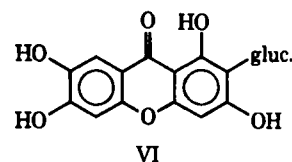
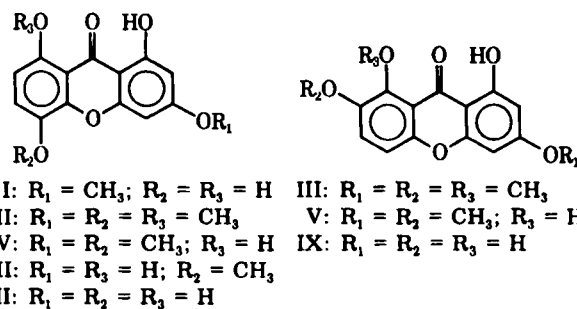
tion. The alcohol-soluble fraction, on concentration, afforded pale-yellow needles, m.p. 263–265° [lit. (12) m.p. 263–264°];  $R_f$  0.48 (Solvent 2);  $\nu_{\text{max}}$  (mineral oil): 3435, 3082, 1652, 1630, 1610, 1592, 1508, 1262, 1240, 1202, 1180, and 1150  $\text{cm}^{-1}$ .

The 3,8-dimethyl ether, prepared with ethereal diazomethane, crystallized from ethanol as light-yellow needles, m.p. and mixed m.p. with xanthone II 205°.

**Polyhydroxyxanthoness (Xanthone VIII and Xanthone IX)**—The chloroform-insoluble solid (Fraction C) (1.34 g.) showed several spots on TLC plates (Solvent 4). Attempts to separate them by preparative layer chromatography failed. A portion of the solid (about 0.2 g.) was methylated with ethereal diazomethane. The ether-soluble fraction, consisting of a mixture of methyl ethers, was subjected to preparative layer chromatography using Solvent 3 as the developer when two major zones were separated. The upper zone afforded 1-hydroxy-3,5,8-trimethoxyxanthone (II), while the lower zone gave 1-hydroxy-3,7,8-trimethoxyxanthone (III). No spot corresponding to 1-hydroxy-3,6,7-trimethoxyxanthone or the methyl ethers of 1,3,5- or 1,3,7-trioxygenated xanthoness could be detected in the presence of authentic markers. Complete demethylation of the two methyl ethers with hydrobromic acid (48%) afforded the respective tetrahydroxyxanthoness—viz., 1,3,5,8-tetrahydroxyxanthone (VIII) and 1,3,7,8-tetrahydroxyxanthone (IX). The natural mixture of the polyhydroxylated xanthoness, present in Fraction C, was subjected to cochromatography with the two tetrahydroxyxanthoness (VIII and IX) when their presence in the mixture was detected. The appropriate fractions from the natural mixture were separated by preparative chromatography and examined spectrometrically (Tables I–III), which established their identities.

## RESULTS AND DISCUSSION

Nine tetraoxygenated xanthoness (I–IX) were isolated from the aerial parts of *S. chirata* Buch.-Ham. (Gentianaceae). Isolation and purification of the compounds involved solvent extraction, fractional crystallization, repeated column and preparative layer chromatography, and preparation of derivatives where possible. The identity of the xanthoness was established by direct comparison (mixed melting point, co-TLC, and superimposable IR spectra) with authentic reference materials and from spectral (UV, IR, PMR, and mass spectrometry) evidence (Tables I–III). The charac-



terization of the xanthoness is described here in the order of their isolation from the plant extract.

**Xanthone I**—This xanthone,  $\text{C}_{14}\text{H}_{10}\text{O}_6$  ( $M^+$ , 274), m.p. 271°, formed a dimethyl ether with ethereal diazomethane. The UV absorption spectrum of the xanthone (Table I) indicated its close similarity with 1,3,5,8-tetraoxygenated xanthoness (9). The UV spectrum remained unchanged in the presence of sodium acetate. It showed one methoxyl group and four aromatic protons in its PMR spectrum (Table II). The aromatic protons appeared as *meta* and *ortho* split doublets associated with H-2, H-4, H-6, and H-7 protons, respectively. A broad two-proton signal appeared at 13.5 p.p.m. and was assigned to the chelated 1- and 8-OH protons. The mass spectrum of the compound showed, aside from the molecular ion as the base peak, a significant peak due to the fragment ion  $M - 29$  (Table III) associated with the loss of  $\text{CHO}$  from the  $\text{C}_3$ —OH group (10). On the basis of these data, together with its insolubility in 5% aqueous sodium carbonate, xanthone I is identified as 1,5,8-trihydroxy-3-methoxyxanthone.

**Xanthone II**—This xanthone,  $\text{C}_{16}\text{H}_{14}\text{O}_6$  ( $M^+$ , 302), m.p. 205°, formed a monomethyl ether with dimethyl sulfate and potassium carbonate. It remained unchanged upon treatment with ethereal diazomethane. The UV absorption spectrum of the compound indicated its close similarity with 1,3,5,8-tetraoxygenated xanthoness (9). In the PMR spectrum of the xanthone, nine protons due to three methoxyl groups and four aromatic protons appeared as *meta* and *ortho* split doublets due to H-2, H-4, H-6, and H-7 protons, respectively. A one-proton signal appeared at 13.33 p.p.m., which remained unchanged upon treatment with deuterium oxide, and was ascribed to the strongly chelated 1-OH. The mass spectrum of the xanthone showed, aside from the molecular ion peak, significant peaks at  $m/e$  287 ( $M - 15$ ) and 259 ( $M - 43$ ), associated with the losses of the  $\text{CH}_3$  radical and  $\text{C}_6\text{H}_5\text{O}$  complex from the molecular ion. On the basis of these observations, xanthone II is identified as 1-hydroxy-3,5,8-trimethoxyxanthone. This conclusion was established by a direct comparison with an authentic synthetic sample.

**Xanthone III**—This xanthone,  $\text{C}_{16}\text{H}_{14}\text{O}_6$  ( $M^+$ , 302), m.p. 148–149°, was found to be identical with decussatin in all respects (10).

**Xanthone IV**—Xanthone IV,  $\text{C}_{16}\text{H}_{12}\text{O}_6$  ( $M^+$ , 288), m.p. 185–186°, is a dihydroxydimethoxyxanthone since it formed a diacetate and

Table II—PMR Spectral Data<sup>a</sup> of the Xanthoness<sup>b</sup> of *S. chirata*

Xanthone	Chelated Hydroxyl	Methoxyl Protons	H-2	H-4	H-5	H-6	H-7	H-8
I <sup>c</sup>	13.5 (broad)	3.90 (3H)	6.32/6.29	6.58/6.54	—	7.38/7.22	6.75/6.60	—
II <sup>d</sup>	13.33 (1-OH)	4.0–3.95 (9H)	6.48/6.43	6.65/6.60	—	7.40/7.24	6.88/6.72	—
IV <sup>d</sup>	11.96, 11.33 (1- and 8-OH)	4.0–3.95 (6H)	6.42/6.37	6.64/6.60	—	7.40/7.24	6.84/6.68	—
VII <sup>c</sup>	13.45 (broad 1-OH)	3.90 (3H)	6.30/6.26	6.58/6.55	—	7.33/7.17	6.74/6.59	—

<sup>a</sup> The signals (in parts per million) were recorded from tetramethylsilane. <sup>b</sup> PMR data of other xanthoness were previously recorded (10). <sup>c</sup> Solvent dimethyl sulfoxide- $d_6$ . <sup>d</sup> Solvent deuteriochloroform.

Table III—Mass Spectral Data of the Xanthenes<sup>a</sup> of *S. chirata*

Xanthone	Molecular Ion, <i>m/e</i> (%)	Significant Peaks, <i>m/e</i> (%)	Metastable Peaks, <i>m/e</i>	
			Calc.	Found
I	274 (100)	259 (3), 246 (7), 245 (17), 231 (14), 217 (8), 216 (7)	$274 \xrightarrow{m^+} 245$ 219;	219
II	302 (100)	287 (18), 273 (7), 259 (8), 231 (3)	$302 \xrightarrow{m^+} 287$ 272.7;	273
IV	288 (100)	273 (22), 258 (5), 245 (7), 230 (16)	$288 \xrightarrow{m^+} 273$ 258.7;	259
VII	274 (100)	259 (14), 231 (18), 203 (8)	$274 \xrightarrow{m^+} 259$ 244.8;	245
VIII	260 (100)	242 (8), 232 (7), 231 (18), 203 (6)		

<sup>a</sup> Mass spectral data of other xanthenes were previously recorded (10).

a monomethyl ether (CH<sub>2</sub>N<sub>2</sub>). The UV spectrum of the xanthone showed its close similarity with 1,3,5,8-tetraoxygenated xanthenes (9). The UV spectrum remained unchanged in the presence of sodium acetate. It showed two methoxyl groups and two *meta* and two *ortho* split doublets in its PMR spectrum. These data and its insolubility in 5% sodium carbonate located one of the methoxyl groups at C<sub>3</sub>. The abundance of the *M* - 15 and *M* - 43 peaks in the mass spectrum of the xanthone (Table III) is consistent (10) with the location of the other methoxyl group at C<sub>5</sub>. The monomethyl ether of the xanthone was identical with xanthone II. On the basis of these observations, xanthone IV is identified as 1,8-dihydroxy-3,5-dimethoxyxanthone.

**Xanthone V**—This xanthone, C<sub>14</sub>H<sub>12</sub>O<sub>8</sub> (*M*<sup>+</sup>, 288), m.p. 175–176°, is a dihydroxydimethoxyxanthone. Its UV absorption spectrum was similar to those of 1,3,7,8-tetraoxygenated xanthenes (10, 12). The UV spectrum remained unchanged in the presence of sodium acetate. The PMR spectrum of the xanthone showed two methoxyl groups and four aromatic protons associated with H-2, H-4, H-5, and H-6 (10). The mass spectrum of the xanthone indicated that

the two methoxyl groups are at the C<sub>3</sub> and C<sub>7</sub> positions (10). This conclusion was confirmed by direct comparison of its monomethyl ether with decussatin (10); they were identical. Xanthone V is, therefore, identified as 1,8-dihydroxy-3,7-dimethoxyxanthone.

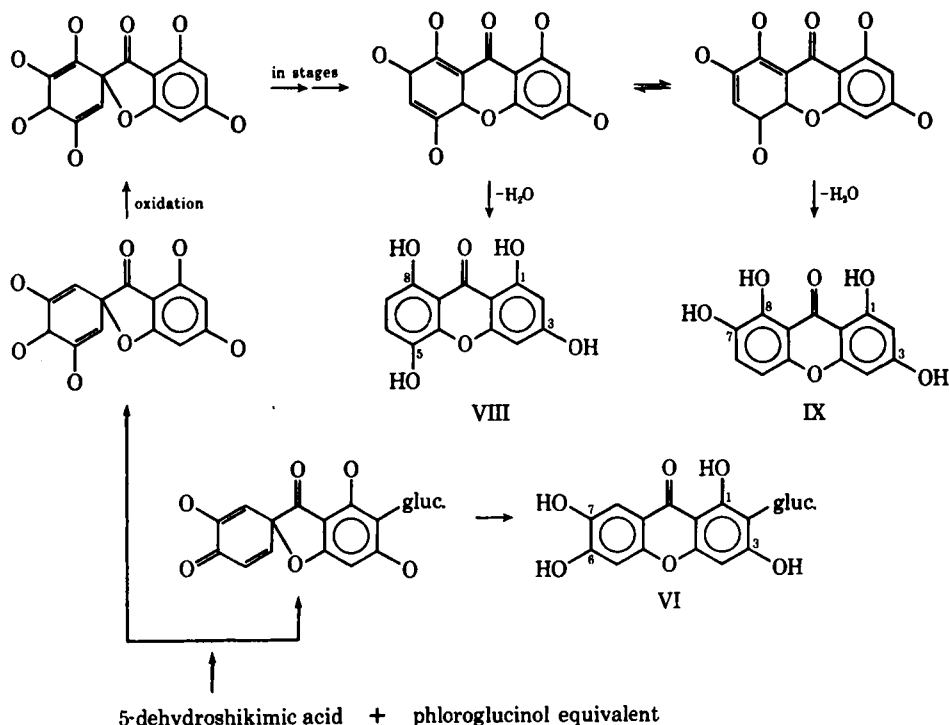
**Xanthone VI**—Xanthone VI, C<sub>18</sub>H<sub>18</sub>O<sub>11</sub> (*M*<sup>+</sup>, 422), m.p. 270–271°, was identical with mangiferin in all respects (10).

Despite several reports in the literature (11, 12, 14) that mangiferin gave an octaacetate under certain experimental conditions, the present investigation conclusively proved that the acetyl derivative formed, under both basic and acidic conditions, was only the heptaacetate. Furthermore, no trace of isomangiferin, which is commonly known to accompany mangiferin in nature (13), was observed in *S. chirata*.

**Xanthone VII**—This xanthone, C<sub>14</sub>H<sub>10</sub>O<sub>8</sub> (*M*<sup>+</sup>, 274), m.p. 263–265°, is a trihydroxymonomethoxyxanthone since it formed a dimethyl ether with ethereal diazomethane and showed one chelated hydroxyl, one methoxyl, and four aromatic protons in its PMR spectrum. The UV spectrum of the xanthone was closely similar to those of 1,3,5,8-tetraoxygenated xanthenes. There was a bathochromic shift of the longer wavelength maximum by about 20 nm. in the presence of sodium acetate. The xanthone was also soluble in 5% aqueous sodium carbonate. These properties are consistent with a free C<sub>2</sub>-OH group in the molecule. The xanthone exhibited abundant fragment ions at *m/e* 259 (*M* - 15) and 231 (*M* - 43) in its mass spectrum (Table III), which located the methoxyl group at C<sub>3</sub>. On the basis of these data, xanthone VII is identified as 1,3,8-trihydroxy-5-methoxyxanthone.

**Xanthenes VIII and IX**—The tetrahydroxyxanthenes (VIII and IX) could not be separated from the mixture of polyhydroxyxanthenes. Methylation with diazomethane and preparative chromatography provided separation of the corresponding trimethyl ethers, which were identified as 1-hydroxy-3,5,8-trimethoxyxanthone and 1-hydroxy-3,7,8-trimethoxyxanthone by direct comparison with reference samples. Complete demethylation of the methyl ethers afforded the tetrahydroxyxanthenes as the pure entities. The natural mixture of the polyhydroxyxanthenes from *S. chirata* was shown to contain these components by co-TLC and preparative layer chromatography. Comparison of the spectral properties of these compounds finally established their structures as 1,3,5,8-tetrahydroxyxanthone (VIII) and 1,3,7,8-tetrahydroxyxanthone (IX).

This study was the first demonstration of the occurrence of 1-hydroxy-3,5,8-trimethoxyxanthone (II) in nature and of mangiferin



Scheme II—Spiran intermediates in the biogenesis of tetraoxygenated xanthenes of *S. chirata*

(VI) in the genus *swertia*. Mangiferin was encountered only once before in this family (Gentianaceae) in *Canscora decussata* Schult (10), along with other polyoxygenated xanthenes (14). This finding is of considerable systematic significance since mangiferin has a uniquely interesting taxonomic character; both in its distribution and biogenesis, it seems to be more closely related to the flavonoids than to other xanthone derivatives (5).

The roots of *S. chirata* contained the same tetraoxygenated xanthenes as in the aerial parts, but the content of mangiferin was less in these parts. No qualitative nor quantitative difference was observed in the xanthonic constituents in *S. chirata* plants collected from Nepal and India. The content of mangiferin declined upon preservation of the plants.

Despite the abundance of the tetraoxygenated xanthenes in *S. chirata*, the corresponding "standard" 1,3,5- and 1,3,7-trioxygenated patterns are completely missing from this plant. In the review of Carpenter *et al.* (5), *S. chirata* was wrongly reported to contain 1,3,7-trioxygenated xanthenes, since in the original paper by Dalal and Shah (4) the plant was reported to contain only 1,8-dihydroxy-3,5-dimethoxyxanthone. In other members of the genus *swertia*, no trioxygenated xanthone was encountered (15). This is particularly germane to the biosynthesis of the 1,3,5,8- and 1,3,7,8-tetraoxygenated xanthenes of *swertia* plants. The tetraoxygenated xanthenes are envisioned as arising from the standard trioxygenated (1,3,5- and 1,3,7-) xanthenes by further oxidation at one of the activated sites ( $C_6$  or  $C_8$ ). The absence of the trioxygenated xanthenes and also of the 1,3,5,6-tetraoxygenated patterns in the members of *swertia* returns the question to a stand-off. It remains to be seen whether these compounds actually represent different modes of cyclization in different taxa, *e.g.*, the coupling route (18, 19) or the spiran intermediate proposed by Gottlieb (20), or whether there is a single path clouded by branching and/or adventitious oxidation. A probable route, involving spiran intermediates, is proposed for the genesis of the tetraoxygenated xanthenes and of mangiferin present in *S. chirata*. The general scheme (Scheme II) could also account for the genesis of mangiferin and the more complex polyoxygenated xanthenes co-occurring in *C. decussata* (10, 16).

In addition to the tetraoxygenated xanthenes, several heterosides, triterpenes, and monoterpene alkaloids were isolated (Scheme I) from the roots and the aerial parts of *S. chirata*. Structure elucidation of these constituents is currently underway.

The results of detailed pharmacological screening of mangiferin was previously reported (21). Preliminary screening of the total xanthenes of *S. chirata* showed definite signs of CNS depression in albino mice and rats. The effect was manifested by initial transient hyperactivity followed by moderate to deep depression, potentiation of hexobarbital sleeping time (by about 145%), and antagonism to 5-methoxy-*N,N*-dimethyltryptamine (22). Hydrocholeretic, cardiostimulant, and anticonvulsant effects were also observed. These activities were determined according to the methods reported earlier (21). The percentage protection offered by the total xanthenes of *S. chirata* against convulsions produced by the administration of pentylenetetrazol was taken as the anticonvulsant activity and was found to be about 65%. The total heterosides showed only feeble activities with these parameters. These observations seem to indicate that the curative properties ascribed to the *S. chirata* plant extracts, in the Indian system of medicine (1, 2), are due to the contained tetraoxygenated xanthenes and their *O*-glucosides.

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