Chemical Constituents of Gentianaceae IV: New Xanthones of *Canscora decussata*

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Abstract □ Three new naturally occurring xanthones—*viz.*, 1-hydroxy-3,5,6-trimethoxyxanthone, 1,6-dihydroxy-3,5-dimethoxyxanthone, and 1,3,6-trihydroxy-5-methoxyxanthone, were isolated from the roots of *Canscora decussata* Schult (family Gentianaceae). The identity of these xanthones was established by chemical reactions and spectral (UV, IR, NMR, and mass spectra) evidence. Phylogenetic significance of the cooccurrence of these and other polyoxygenated xanthones in *C. decussata* is discussed.

Keyphrases □ Xanthones, 1-hydroxy-3,5,6-trimethoxy-, 1,6-dihydroxy-3,5-dimethoxy-, and 1,3,6-trihydroxy-5-methoxy—isolation and identification from *Canscora decussata* □ *Canscora decussata* Schult (Gentianaceae)—isolation and identification of three new xanthones □ Gentianaceae—chemical constituents, isolation and identification of three new xanthones from *Canscora decussata* □ Medicinal plants—*Canscora decussata*, isolation and identification of three new xanthones

The occurrence of polyoxygenated xanthones in the different parts of *Canscora decussata* Schult (family Gentianaceae) was recently reported from this laboratory (1, 2). Further chemical investigation with the roots of this plant resulted in the isolation of three more new xanthones. Characterization of these xanthones and the chemotaxonomic significance of the cooccurrence of several polyoxygenated xanthones and a xanthone C-glucoside are reported in this paper. *C. decussata* is used in the Ayurvedic system of medicine for a variety of purposes (3). Since xanthones are the major constituents of this plant, pharmacological screening of these compounds was also performed to rationalize the uses of the plant extracts in the indigenous system of medicine. The findings of pharmacological studies were previously reported (4).

EXPERIMENTAL[1]

Extraction of *C. decussata* Roots[2]—Dried and milled roots (2.1 kg.) were continuously extracted (Soxhlet) (16 hr.) with petroleum ether (60–80°). The solvent was removed from the petroleum ether extract under reduced pressure when an amorphous residue (161 g.) was obtained. The residue dissolved in diethyl ether (100 ml.), and the solution was extracted with aqueous sodium hydroxide (5%, four 25-ml. portions). The aqueous layer was cooled and acidified with concentrated hydrochloric acid, and the acidic solution was extracted with chloroform (four 30-ml. portions). The combined chloroform extract was washed with water, dried (anhydrous calcium chloride), and concentrated to a small volume (about 15 ml.). The chloroform concentrate was chromatographed over silica gel (200 g.). Elution was done with petroleum ether (60–80°, 2 l.) and benzene (5 l.). The eluates, upon evaporation, gave light-yellow amorphous solids (0.51 and 1.42 g., respectively).

The solid obtained from the petroleum ether fraction showed several spots on TLC plates, but repeated column chromatography failed to separate any individual xanthone. The mixture of xanthones remained unchanged upon treatment with dimethyl sulfate and potassium carbonate, indicating that they are permethylated.

The solid obtained from the benzene fraction showed several spots on TLC plates. It was dissolved in chloroform (8 ml.) and chromatographed over silica gel (100 g.). Benzene, chloroform, and different proportions of mixtures thereof were used as eluents.

Xanthone A (1-Hydroxy-3,5,6-trimethoxyxanthone)—Early benzene eluates afforded 1-hydroxy-3,5-dimethoxyxanthone (300 mg.), m.p. 178–179° (mixed melting point, co-TLC, and superimposable IR) (1). Later benzene eluates gave a mixture of xanthone A (major component) and 1-hydroxy-3,5-dimethoxyxanthone. The major component was purified by rechromatography. It crystallized from ethanol as light-yellow needles (92 mg.), m.p. 179–181°; mixed melting point with an authentic synthetic sample, m.p. 179–181° [prepared according to the method of Shah and Shah (5) and also from maclurin] remained undepressed.

Table I—Mass Spectral Data of the Xanthones of *C. decussata* Roots

Xanthone	Molecular Ion, *m/e* (%)	Significant Peaks, *m/e* (%)	Metastable Peaks, *m/e* Calc.	Found
A	302 (100)	287 (22); 273 (22); 272 (11); 259 (65)	302 → 287	272.7
			302 → 273	246.4
B	288 (100)	273 (9); 259 (13); 258 (12); 245 (22)	288 → 273	258.4
			288 → 259	232.9
C	274 (100)	259 (10); 245 (38); 244 (12); 231 (7)	274 → 259	244.8
			274 → 245	219.0

Table II—UV Absorption Maxima of the Xanthones of *C. decussata* Roots

Xanthone	λ_{max}, nm. (log ϵ) in Ethanol
A	245 (4.67), 282[a] (4.03), 314 (4.37), 338 (3.97)
B	225 (4.50), 240 (4.47), 280[a] (3.92), 315 (4.30)
C	205 (4.39), 220 (3.94), 248 (4.72), 280[a] (4.01), 315 (4.24), 332 (4.08)

[a] Inflection.

[1] All melting points were determined on a Toshniwal melting-point apparatus in open capillaries and are uncorrected. UV spectra were determined in aldehyde-free ethanol on a Carl-Zeiss spectrophotometer. IR spectra were determined on a Perkin-Elmer 237 instrument in KBr pellets, unless otherwise stated.

NMR spectra were run in $CDCl_3$ or dimethyl sulfoxide-d_6 on a Varian A-60D instrument. Mass spectra were recorded on a A.E.I. MS-9 double-focusing spectrometer with an ionizing potential of 70 ev.; samples were directly inserted on a probe. Separation by column chromatography was carried out using silica gel (British Drug Houses, 60–120 mesh). TLC experiments were done with Kiesel-G (E. Merck).

[2] The plant material was supplied by Mr. B. Singh, Varanasi, India, and a herbarium specimen has been preserved at the Botany Department, Banaras Hindu University, Varanasi, India.

Table III—NMR Data[a] of the Xanthones of *C. decussata* Roots

Xanthone	Methoxyl Protons	H-2	H-4	H-7	H-8
A[b]	3.95–4.0 (9H)	6.35/ 6.31	6.58/ 6.53	7.03/ 6.87	8.08/ 7.92
B[c]	3.89–3.95 (6H)	6.34/ 6.30	6.62/ 6.58	7.06/ 6.90	8.04/ 7.88
C[c]	3.85 (3H)	6.28/ 6.23	6.53/ 6.48	6.98/ 6.82	7.63/ 7.47

[a] The signals (in parts per million) were recorded from tetramethylsilane. [b] Deuteriochloroform as solvent. [c] Dimethyl sulfoxide-d_6 as solvent.

The 1-acetyl derivative of xanthone A crystallized from acetone as colorless needles, m.p. and mixed m.p. 147–148°.

The 1-methyl ether was prepared with dimethyl sulfate and potassium carbonate in dry acetone under reflux conditions (46 hr.). It crystallized from ether–petroleum ether (1:1) as colorless needles, melting point and mixed melting point with an authentic synthetic specimen 146–148°.

Xanthone B (1,6-Dihydroxy-3,5-dimethoxyxanthone)—Early benzene–chloroform (1:1) eluates afforded a mixture of 1,5-dihydroxy-3-methoxyxanthone, m.p. 272° (mixed melting point, co-TLC, and superimposable IR) (1) and xanthone B. These were separated by fractional crystallization from ethanol, in which 1,5-dihydroxy-3-methoxyxanthone was sparingly soluble. Xanthone B, obtained from the alcoholic mother liquor, recrystallized from alcohol–petroleum ether (1:1) as pale-yellow needles, m.p. 192–193°.

The 6-*O*-methyl ether was prepared by treatment of xanthone B with ethereal diazomethane. The derivative crystallized from ethanol as yellow needles, m.p. 179–181°. Mixed melting point with 1-hydroxy-3,5,6-trimethoxyxanthone remained undepressed.

Xanthone C (1,3,6-Trihydroxy-5-methoxyxanthone)—The chloroform eluates afforded xanthone C as a brown microcrystalline solid (32 mg.), m.p. 285–289°. Xanthone C crystallized from methanol as light-yellow needles, m.p. 290–291°.

The 3,6-di-*O*-methyl ether, prepared with ethereal diazomethane, crystallized from ethanol as yellow needles, m.p. 181°. It was identical with xanthone A.

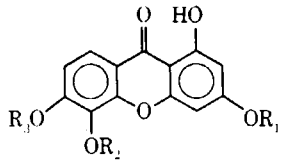
The mass, UV, and NMR spectral data of the three xanthones (A–C) are recorded in Tables I–III, respectively.

RESULTS AND DISCUSSION

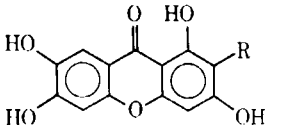
Three previously unreported tetraoxygenated xanthones (xanthones A–C) were isolated from the petroleum ether extract of the roots of *C. decussata*, and their identity was established by chemical reactions and spectral evidence (Tables I–III). In addition to the three new naturally occurring xanthones, two previously reported xanthones—*viz.*, 1-hydroxy-3,5-dimethoxyxanthone and 1,5-dihydroxy-3-methoxyxanthone (1), together with a number of permethylated unidentified xanthones were isolated from the petroleum ether extract. The characterization of the new xanthones is described here in the order of their isolation.

Xanthone A, $C_{16}H_{14}O_6$ (M^+, 302), m.p. 179–181°, formed a monoacetate and a monomethyl ether (with dimethyl sulfate and alkali). It remained unchanged upon treatment with ethereal diazomethane. The UV absorption spectrum of the compound indicated its close similarity with 1,3,5,6-tetraoxygenated xanthones (1). Xanthone A showed three methoxyl groups and four aromatic protons in its NMR spectrum. The aromatic protons appeared as *meta* and *ortho* split doublets associated with H-2, H-4, and H-7, H-8 protons, respectively. A one-proton singlet appeared at δ 12.97 and was ascribed to the strongly chelated 1-OH. The signal remained unchanged upon treatment of the xanthone with deuterium oxide. On the basis of these observations, xanthone A was identified as 1-hydroxy-3,5,6-trimethoxyxanthone. This conclusion was further confirmed by a direct comparison of the xanthone with an authentic synthetic specimen of 1-hydroxy-3,5,6-trimethoxyxanthone (5).

Xanthone B, $C_{15}H_{12}O_6$ (M^+, 288), m.p. 192–193°, is a dihydroxydimethoxyxanthone; it formed a diacetate and a monomethyl ether (diazomethane). The methyl ether was identical with 1-hydroxy-3,5,6-trimethoxyxanthone. The UV absorption and NMR spectra of xanthone B also showed its close similarity with 1,3,5,6-tetraoxygenated xanthones. Its insolubility in aqueous sodium carbonate and unchanged UV maxima in ethanolic sodium acetate (6) indicated that a methoxyl group is at C-3. The abundance of the M − 15 peak (fragment ion at *m/e* 273) in its mass spectrum locates the methoxyl group at C-5. Xanthones with a 5-methoxy substituent are known (1, 7, 8) to produce abundant fragment ions corresponding to the loss of a methyl radical, while for *m*-methoxyphenols (equivalent to a C-6 methoxyl in xanthones) virtually no M − 15 peak has been found (9). Similar anticipated peaks were observed in the current studies with other methoxylated xanthones.



xanthone A: $R_1 = R_2 = R_3 = CH_3$
xanthone B: $R_1 = R_2 = CH_3$, $R_3 = H$
xanthone C: $R_1 = R_3 = H$, $R_2 = CH_3$



mangiferin
(R = β-D-glucoside)

Xanthone C, $C_{14}H_{10}O_6$ (M^+, 274), m.p. 290–291°, was previously obtained (1) from the alcoholic extracts of *C. decussata* in appreciable quantities but could not be completely characterized at that time. It formed a dimethyl ether with diazomethane and a trimethyl ether with dimethyl sulfate and alkali. The UV and NMR spectra of the xanthone are characteristic of a 1,3,5,6-tetraoxygenated xanthone. The dimethyl ether was identical with 1-hydroxy-3,5,6-trimethoxyxanthone. The position of the only methoxyl group in xanthone C was determined on the basis of: (*a*) its solubility in aqueous sodium carbonate, (*b*) its failure to give Tollen's test, (*c*) its unchanged UV absorption maxima in ethanolic sodium acetate–boric acid, and (*d*) the shift in its major UV absorption peaks in the presence of a trace of sodium acetate. These observations locate the methoxyl group of xanthone C at either C-5 or C-6 but not at C-3. The facile loss of 15 mass units from its molecular ion peak (M^+) indicated that the methoxyl group is at C-5.

This is the first report of the occurrence of 1,3,5,6-tetraoxygenated xanthones in the family Gentianaceae and of xanthones A–C in nature. Also, the cooccurrence of 1,3,5,6-tetraoxygenated xanthones with the free 1,3,6,7-tetrahydroxyxanthone (8) and 1,3,6,7-tetrahydroxyxanthone C_2-β-D-glucoside, mangiferin, in a single plant species (*C. decussata*), may have phylogenetic and biogenetic significance, since these two oxygenated patterns (1,3,5,6 and 1,3,6,7) are reported to be derived from a common benzophenone intermediate, maclurin, by phenolic oxidation (10).

REFERENCES

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

(2) S. Ghosal, R. K. Chaudhuri, and A. Nath, *J. Indian Chem. Soc.*, **48**, 589(1971).

(3) R. N. Chopra, S. L. Nayar, and I. C. Chopra, "Glossary o. Indian Medicinal Plants," C.S.I.R., New Delhi, India, 1956, p. 49f

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

(5) G. D. Shah and R. C. Shah, *J. Sci. Ind. Res.*, **15B**, 630(1956).

(6) D. De Barros Correa, L. G. Fonseca, E. Silva, O. R. Gottlieb, and S. Janot Gonclaves, *Phytochemistry*, **9**, 447(1970).

(7) B. Jackson, H. D. Locksley, and F. Scheinmann, *J. Chem. Soc., C*, **1967**, 785.

(8) S. Ghosal, R. K. Chaudhuri, and S. K. Bhattacharya, *Abstracts 8th IUPAC Symp. Chem. Natural Products, New Delhi*, **1972**, 78.

(9) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Interpretation of Mass Spectra of Organic Compounds," Holden-Day, San Francisco, Calif., 1965, p. 178.
(10) I. Carpenter, H. D. Locksley, and F. Scheinmann, *Phytochemistry*, **8**, 2013(1969).

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Antitumor Agents from *Alnus oregona* (Betulaceae)

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Abstract □ The chloroform extract of *Alnus oregona* showed antitumor activity against the Walker 256 (5WA16) tumor system. Lupeol and betulin were identified as the two constituents responsible for this activity.

Keyphrases □ *Alnus oregona* (Betulaceae)—isolation and identification of two antitumor constituents, lupeol and betulin □ Lupeol—antitumor agent identified from *Alnus oregona* □ Betulin—antitumor agent identified from *Alnus oregona* □ Antitumor activity—isolation and identification of lupeol and betulin as antitumor constituents from *Alnus oregona*

During the routine screening of Southwestern plants for potential antitumor activity, the chloroform extract of the stembark of *Alnus oregona* Nutt. showed significant antitumor activity in Sprague rats against the Walker 256 intramuscular tumor system (5WA16)[1]. Activity in this system is defined as a percent T/C value of less than 60 in a satisfactory dose–response test (1). The plant was collected in California[2].

Triterpenes belonging mainly to the taraxane and lupane series have been isolated from various species of *Alnus*. Taraxerol, taraxerone, lupeol, lupenone, betulin, and betulinic acid as well as other triterpene compounds have been isolated from *A. glutinosa* (2, 3), *A. incana* (4, 5), *A. virdis* (6), *A. barbata* (7), and *A. subcordata* (8). However, a search of the literature failed to reveal any chemical investigation of *A. oregona*.

RESULTS AND DISCUSSION

Because of its chemical complexity, the chloroform extract was separated into six fractions by column chromatography using partially deactivated alumina (Table I). Fraction E, which consisted essentially of β-sitosterol, was not screened further since the Cancer Chemotherapy National Service Center has indicated that this compound showed marginal activity in the 5WA16 tumor system. Only Fractions C and F showed significant activity (Table II).

Fractions C and F contained essentially single components. Fraction C, upon recrystallization from chloroform–methanol, yielded a crystalline compound, m.p. 210–212°. Its mass spectrum showed an M^+ peak at 426 with major fragments at *m/e* 218, 207, and 189. These were indicative of the lupene skeleton (9). The NMR spectrum of the compound indicated vinyl protons at δ 4.66 and 4.75 (d) as well as 3α-H at δ 3.28 (m), further proof that the compound was probably lupeol. Identification was confirmed by melting point, optical rotation, and IR of the compound as well as of the acetate and benzoate. The IR of the latter was superimposable with the IR of an authentic sample of lupeol benzoate[3].

The crystalline compound isolated from Fraction F (m.p. 252–254°) was identified as betulin. The mass spectrum showed an M^+ peak at 442, and the NMR spectrum indicated the appropriate signal for vinyl protons. The IR spectra of the compound and its diacetate were superimposable with authentic samples[4].

Table I—Alumina Chromatography of Crude Extract

Fraction	Eluent		Components
A	Hexane to hexane–benzene	(3:1)	—
B	Hexane–benzene	(6:4)	—
C	Hexane–benzene	(1:1)	Lupeol
D	Hexane–benzene	(1:1)	—
E	Benzene		β-Sitosterol
F	Benzene–chloroforn	(3:1)	Betulin

Table II—Biological Activity against 5WM Tumor System

Compound	Dose, mg./kg.	Survivors	Percent T/C (1)
Crude extract	200	4/4	28
Fraction C	400	4/4	22
	200	4/4	46
Fraction F	400	4/4	39
Lupeol	200	4/4	39
Betulin	600	4/4	13
	400	3/4	26

EXPERIMENTAL

Extraction—Twelve kilograms of the dried stembark of *A. oregona* was extracted with 21 l. of chloroform in an extractor (Lloyd). The extract, after filtration and removal of the chloroform, weighed 382 g.

Column Chromatography—Neutral alumina (3.8 kg., activity III) was packed in a glass column (64 × 10.5 cm.), using *n*-hexane

[1] Cancer Chemotherapy National Service Center, Bethesda, MD 20014

[2] By the U. S. Department of Agriculture.

[3] Obtained through the courtesy of Dr. Jack L. Beal, College of Pharmacy, Ohio State University, Columbus, Ohio.

[4] Authentic specimens of betulin and its diacetate were obtained through the courtesy of Dr. C. Steelink, Department of Chemistry, University of Arizona, Tucson, AZ 85721