Chemical Constituents of Gentianaceae X: Xanthone-*O*-glucosides of *Swertia purpurascens* Wall.

S. GHOSAL ˣ, P. V. SHARMA, and R. K. CHAUDHURI

Abstract □ Three tetraoxygenated xanthone-*O*-glucosides—*viz.*, 3,5,8-trihydroxyxanthone-1-*O*-glucoside (norswertianolin, I), 5,8-dihydroxy-3-methoxyxanthone-1-*O*-glucoside (swertianolin, II), and 1,5-dihydroxy-3-methoxyxanthone-8-*O*-glucoside (III), were isolated from *Swertia purpurascens* Wall. (Gentianaceae). The identity of the compounds was established by chemical transformation and by spectral (UV, IR, NMR, and mass) evidence. Among these compounds, III, named isoswertianolin, was not encountered before in nature or prepared synthetically. Swertianolin (II) was previously isolated from *S. japonica* and *S. tosaensis*, but its structure was only partially defined until this investigation. Selected pharmacological screening of the total xanthone-*O*-glucosides of *S. purpurascens* showed noteworthy CNS depressant, cardiovascular stimulant, and anticonvulsant activities. The pharmacological profile of activities of these compounds indicated that the curative properties ascribed to the plant extracts, in the Indian system of medicine, are essentially due to the xanthone-*O*-glucosides. Biochemical significance of the natural occurrence of xanthone-*O*-glycosides and their therapeutic potentiality were also appraised.

Keyphrases □ *Swertia purpurascens* Wall. (Gentianaceae)—isolation, chemical characterization of three tetraoxygenated xanthone-*O*-glucosides □ Xanthone-*O*-glucosides—isolation from *S. purpurascens* □ Norswertianolin—isolation, identification from *S. purpurascens* □ Swertianolin—isolation, characterization from *S. purpurascens* □ Isoswertianolin—isolation, characterization from *S. purpurascens* □ Gentianaceae—occurrence of xanthone-*O*-glucosides, phylogenetic significance

As part of a search for xanthonic constituents of the various genera of Gentianaceae, *Swertia purpurascens* Wall., collected in flowers from Jogindernagar, Himachal Pradesh, India, was examined. *S. purpurascens* is widely distributed in India in the temperate North Western Himalayas, 1524–3657 m (5000—12,000 ft) from Kashmir to Kumaon, and is used in the Indian system of medicine as a substitute for *S. chirata* Buch.-Ham. (1). Previous phytochemical investigations of *S. purpurascens* were limited to the isolation of oleanolic acid, swertiamarin, and swertisin (2). Several sister species are well known for liberally producing tetraoxygenated xanthones (3, 4). Naturally occurring xanthones were recently shown to produce a number of significant biological activities (4, 5). Mangiferin (1,3,6,7-tetrahydroxyxanthone-C_2-β-D-glucoside), the major xanthone of *Canscora decussata* (Gentianaceae) (6–8) and *S. chirata* (4), showed noteworthy monoamine oxidase inhibitor, cardiovascular stimulant, anticonvulsant, and choleretic activities (4, 5, 9) consistent with the uses of these plant extracts in the Indian system of medicine.

Norswertianolin, isolated from *S. randaiensis*, was reported to produce significant tuberculostatic activity (10). This activity was first reported for 1,3,8-trihydroxyxanthone, a degradation product of sterigmatocystin (11). Interestingly, some medicinal plants used in India as antitubercular vegetable drugs (1, 12) were subsequently found to contain polyoxygenated xanthones as their major chemical constituents (4, 6–8). Synthetic 1,3- and 1,6-dihydroxyxanthones, closely related to the xanthones of *Mammea americana* (Guttifereae), exhibited some degree of growth-inhibiting activity against Sarcoma 180 tumor cells (13). It was, therefore, thought worthwhile to investigate the xanthonic constituents of the title species and to determine the pharmacological profile of their activities. The present paper describes the details of isolation, characterization, and selected pharmacological screening of the xanthone-*O*-glucosides of *S. purpurascens*. Additionally, the biochemical significance and potential importance of the natural xanthone-*O*-glycosides as antipsychotic agents are appraised.

EXPERIMENTAL[1]

Extraction of *S. purpurascens*.—The general procedure followed for the isolation of the xanthone-*O*-glucosides of *S. purpurascens*[2] is shown in Scheme I.

Norswertianolin (I).—The solid, marked Fraction B, crystallized from about 200 ml of methanol–dioxane (1:1) as cream-colored microcrystals, mp 263–266° [lit. (10) mp 263–265°]; $[\alpha]_D^{25}$ −111° (c 0.28, 60% aqueous ethanol); UV: λ_{max} 250 (log ϵ 4.38), 275–280 (4.15), and 325 (3.84) nm; λ_{max} (C_2H_5OH–0.2% NaO-$COCH_3$): 250, 275, and 360 nm; NMR (dimethyl sulfoxide-d_6): δ 7.28 (1H, d, J = 9 Hz, C_6-H), 6.65 (1H, d, J = 9 Hz, C_7-H), 6.58 (1H, d, J = 3 Hz, C_4-H), 6.44 (1H, d, J = 3 Hz, C_2-H), 4.92 (1H, m, C_1-H of β-glucoside), and 3.50 (six glucosyl protons plus water).

Anal.—Calc. for $C_{19}H_{18}O_{11}$·H_2O: C, 51.81; H, 4.09. Found: C, 51.47; H, 4.44.

Norswertianolin Heptaacetate—Norswertianolin (24 mg), on treatment with acetic anhydride (1 ml) and pyridine (2 drops), gave the heptaacetate which crystallized from hexane–methylene chloride as colorless needles, mp 222–224°.

Hydrolysis of Norswertianolin—Norswertianolin (48 mg) was hydrolyzed with sulfuric acid (3%, 10 ml) for 2 hr on a steam bath. Extraction with ethyl acetate, evaporation of the solvent, and crystallization of the residue from methanol gave 1,3,5,8-tetrahydroxyxanthone, which had a melting point and mixed melting point of 295–298° (4). The presence of glucose in the aqueous hydrolysate

[1] Melting points were determined on a Toshniwal apparatus in open capillaries and are uncorrected. UV spectra were determined with 95% aldehyde-free ethanol, unless stated otherwise, on a Cary 14 or Spektromom 203 spectrophotometer. IR spectra were determined on a Perkin-Elmer 237 instrument in KBr pellets. NMR spectra were run on a Varian A-60 D or XL-100 spectrometer in $CDCl_3$ or dimethyl sulfoxide-d_6 using tetramethylsilane as an internal standard. Mass spectra were recorded with an AEI MS9 double-focusing spectrometer with an ionizing potential of 70 ev. Silica gel G (E. Merck) was used for analytical and preparative TLC (plate thickness 0.25 and 2 mm); iodine vapor was used for staining.

[2] The plant material was collected by Mr. M. R. Uniyal, Survey of Indian Medicinal Plants Unit, Central Council of Research in Indian Medicine and Homeopathy, Jogindernagar, Himachal Pradesh. A voucher specimen is available in the Department of Pharmaceutics, Banaras Hindu University, Varanasi-5, India.

was detected by partition paper chromatography[3] using *n*-butanol–acetic acid–water (4:1:5) as the developer, R_f 0.20.

Permethylation of Norswertianolin—Norswertianolin (56 mg) was methylated with dimethyl sulfate (2 ml) and potassium carbonate (1 g) in anhydrous acetone (50 ml) for 46 hr on a steam bath. The heptamethyl ether crystallized from methanol as yellow needles, mp 188–190°.

Hydrolysis of Norswertianolin Heptamethyl Ether—The heptamethyl ether (42 mg) was hydrolyzed with dilute sulfuric acid (3%, 10 ml) in the usual way. Extraction of the aglucone with ether, evaporation of the solvent, and crystallization of the residue from benzene gave 1-hydroxy-3,5,8-trimethoxyxanthone, which had a melting point and mixed melting point of 204–205°.

Swertianolin (II)—The first crop from Fraction C was obtained as pale-yellow needles (marked Fraction D). It showed one major spot and one minor spot on TLC. Repeated crystallization from methanol–dioxane gave the major product as a homogeneous entity, mp 204–205° [lit. (14, 15) mp 208 and 226°, respectively]; $[\alpha]_D^{25}$ −115° (c 0.31, 60% aqueous ethanol) [lit. (15) $[\alpha]_D^{18}$ −112.27°]; UV: λ_{max} 252 (log ϵ 4.40), 275 (4.22), and 325 (3.95) nm; NMR (dimethyl sulfoxide-d_6): δ 12.95 (1H, br s, C_8-OH), 7.30 (1H, d, J = 9 Hz, C_6-H), 6.68 (1H, d, J = 9 Hz, C_7-H), 6.60 (1H, d, J = 3 Hz, C_4-H), 6.52 (1H, d, J = 3 Hz, C_2-H), 5.0 (1H, m, C_1-H of β-glucoside), and 3.50 (six glucosyl protons plus water).

Anal.—Calc. for $C_{20}H_{20}O_{11}\cdot H_2O$: C, 52.86; H, 4.40. Found: C, 52.42; H, 4.76.

Swertianolin Hexaacetate—Swertianolin (28 mg) was acetylated with acetic anhydride (1 ml) and pyridine (2 drops) on a steam bath for 2 hr. The hexaacetate crystallized from hexane–methylene chloride as colorless needles, mp 226–228° [lit. (14) mp 226°].

Hydrolysis of Swertianolin—Swertianolin (42 mg) was hydrolyzed with dilute sulfuric acid (3%, 10 ml) in the usual way. Extraction with ethyl acetate, evaporation of the solvent, and crystallization of the residue from methanol afforded 1,5,8-trihydroxy-3-methoxyxanthone (bellidifolin), as shown by the melting point and mixed melting point of 270–271° and co-TLC (4). Presence of glucose in the hydrolysate was established by partition paper chromatography. The hydrolysate was evaporated *in vacuo* to a syrup, which was subjected to partition paper chromatography by a double-ascending method using *n*-butanol–pyridine–water (3:2:1 v/v) as the developer and *p*-anisidine hydrochloride as the staining reagent. The chromatogram showed a single spot (yellowish brown) identical with D-glucose run in parallel.

Swertianolin Dimethyl Ether—Swertianolin (98 mg) was methylated according to the method of Nakaoki and Hida (15). The product showed one major and two minor spots on TLC. The major component was separated by preparative TLC [chloroform–acetic acid (100:5) was used as the developer] as a yellow amorphous solid. It was subjected to hydrolysis without further purification.

Hydrolysis of Swertianolin Dimethyl Ether—The above compound (78 mg) was hydrolyzed with sulfuric acid (3%, 20 ml). Extraction of the aglucone with ether, evaporation of the solvent, and crystallization of the residue from ethanol afforded 1-hydroxy-3,5,8-trimethoxyxanthone, which had a melting point and mixed melting point of 204–205°.

Isoswertianolin (III)—The solid, marked Fraction E, crystallized from methanol as pale-yellow needles, mp 220–222°; $[\alpha]_D^{25}$ −110 ± 2° (c 0.25, 60% aqueous ethanol); UV: λ_{max} 252 (log ϵ 4.43), 275 (4.18), and 325 (3.94) nm; NMR (dimethyl sulfoxide-d_6): δ 13.18 (1H, br s, C_1-OH), 7.15 (1H, d, J = 9 Hz, C_7-H), 7.33 (1H, d, J = 9 Hz, C_6-H), 6.55 (1H, d, J = 3 Hz, C_4-H), 6.38 (1H, d, J = 3 Hz, C_2-H), 4.98 (1H, m, C_1-H of β-glucoside), and 3.50 (six glucosyl protons plus water).

Anal.—Calc. for $C_{20}H_{20}O_{11}\cdot H_2O$: C, 52.86; H, 4.40. Found: C, 52.49; H, 4.66.

Isoswertianolin Dimethyl Ether—To a solution of isoswertianolin (198 mg) in dimethylformamide (5 ml) and methanol (50 ml) was added an ethereal solution of diazomethane, prepared from nitrosomethylurea (2 g). The reaction was kept at room temperature (10–24°) overnight. Then the solvent was removed and the residue was triturated with methanol (2 ml) when a pale-yellow crystalline product (148 mg) was obtained. It showed one major and two minor spots on TLC [chloroform–methanol–water (7:3:1) lower layer]. The major component, being most polar, was separated by preparative TLC. The minor components could be due to partial methylation of the sugar moiety in III. The major component crystallized from ethanol as yellow needles, mp 178–180°; UV: λ_{max} 235 (log ϵ 4.30), 275–278 (3.94), 300 (3.90), 315 (3.82), and 360–365 (3.40) nm.

Hydrolysis of Isoswertianolin Dimethyl Ether—To a solution of emulsin (108 mg) in acetate buffer (pH 4.9, 20 ml), isoswertianolin dimethyl ether (98 mg) was added. The mixture was kept at 37° for 24 hr. The solution was then extracted with ether and the ether extract was processed in the usual way to give the aglucone as fine yellow needles (42 mg). Presence of glucose in the hydrolysate was established by partition paper chromatography.

1,3,5-Trimethoxy-8-hydroxyxanthone—The aglucone was crystallized from benzene as fine needles, mp 210–212°. It showed a depression in the melting point when admixed with authentic 1-hydroxy-3,5,8-trimethoxyxanthone, mp 204–205°. Co-TLC [chloroform–benzene (1:1)] also indicated that they were different. This new xanthone showed UV: λ_{max} 225 (log ϵ 4.02), 230–238 (4.17), 252 (4.38), 275–278 (3.90), and 330 (3.86) nm; NMR ($CDCl_3$): δ 13.30 (1H, s, C_8-OH), 7.38 (1H, d, J = 10 Hz, C_6-H), 6.80 (1H, d, J = 10 Hz, C_7-H), 6.64 (1H, d, J = 3 Hz, C_4-H), 6.42 (1H, d, J = 3 Hz, C_2-H), and 4.00–3.95 (9H, OCH_3); mass spectrometry: *m/e* 302 (M^+, 88%) and significant fragment ion peaks at *m/e* 287 (100), 272 (12), 259 (24), and 230 (8), consistent with the assigned structure.

Complete methylation of this compound with dimethyl sulfate and potassium carbonate in anhydrous acetone for 48 hr on a steam bath gave 1,3,5,8-tetramethoxyxanthone, which had a melting point and mixed melting point of 208–209° (4). Co-TLC [chloroform–benzene (1:1)] showed a single spot having the same R_f value and the IR spectra were superimposable.

RESULTS AND DISCUSSION

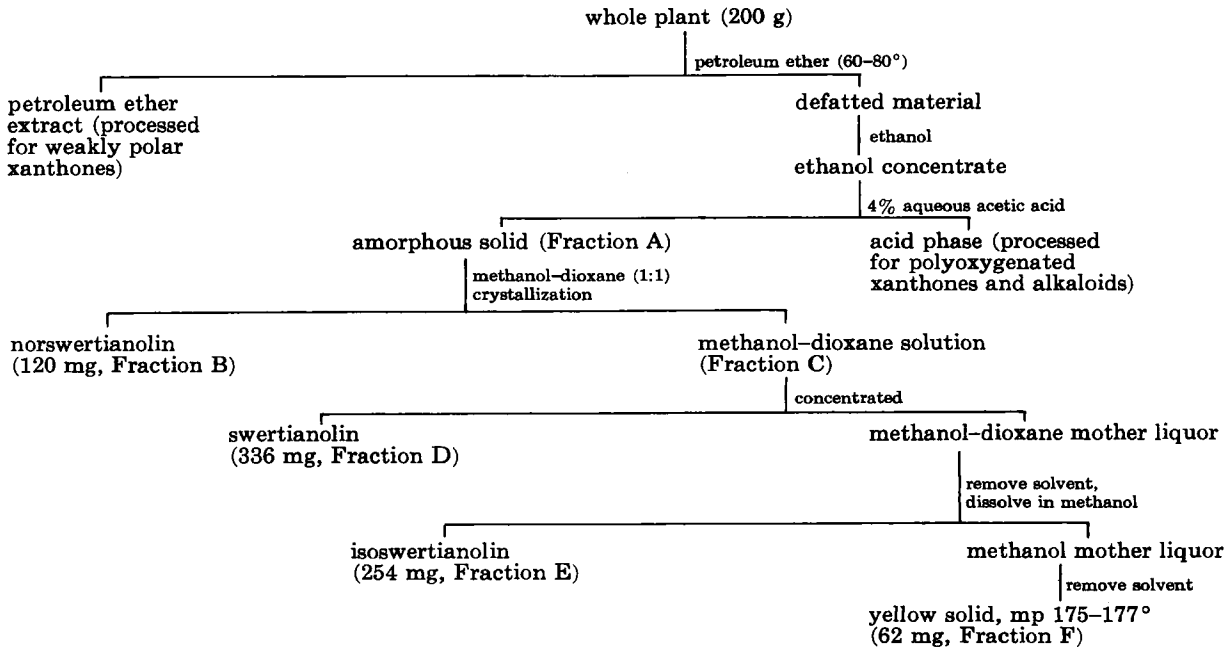
Three xanthone-*O*-glucosides were isolated from the alcoholic extract of *S. purpurascens*. The compounds were characterized by chemical transformation, such as derivatization, hydrolysis into xanthone and glucose, direct comparison of the two moieties so fragmented with reference materials where possible, and spectral (UV, IR, NMR, and mass) evidence. The characterization of these compounds will be described in order of isolation (Scheme I).

Xanthone-*O*-glucoside (I)—Compound I had the following characteristics: $C_{19}H_{18}O_{11}\cdot H_2O$, mp 263–266°, and $[\alpha]_D^{25}$ −111°; it formed a heptaacetate. The UV absorption spectrum showed close similarity with spectra of 1,3,5,8-tetraoxygenated xanthones (4, 16). In ethanolic sodium acetate, the UV spectrum showed a pronounced bathochromic shift of the longest wavelength maximum (λ 325 → 360 nm), indicating a free C_3-OH. The compound also responded to a quinol test (17), suggestive of the presence of free C_5- and C_8-OH groups. In the NMR spectrum, the compound showed signals due to four aromatic protons (δ 7.3–6.4), seven protons associated with glucosyl linkage (δ 4.9, 3.5) (18), and a strongly deshielded proton associated with a chelated OH. The aromatic protons appeared as *meta*- and *ortho*-split doublets associated with C_2-, C_4-, C_6-, and C_7-protons. The glucose anomeric proton showed a diaxial coupling with the $C_{2'}$-H, suggesting a β-glucosidic linkage (18) in the xanthone-*O*-glucoside. The strongly deshielded proton is ascribed to C_8-OH (6, 19). On hydrolysis with dilute sulfuric acid, it gave 1,3,5,8-tetrahydroxyxanthone (4) and glucose.

On the basis of these data, the xanthone-*O*-glucoside was identified as 3,5,8-trihydroxyxanthone-1-*O*-glucoside (I) (norswertianolin). Norswertianolin was previously isolated from *S. randaiensis* (10), but its detailed spectral data were not reported. It was reported to show noteworthy tuberculostatic activity (10). The tuberculostatic activity ascribed to *Swertia* plant extracts (1, 12) could be due to norswertianolin and the congener xanthone-*O*-glucosides.

Xanthone-*O*-glucoside (II)—Compound II had the following characteristics: $C_{20}H_{20}O_{11}\cdot H_2O$, mp 204–205°, and $[\alpha]_D^{25}$ −115°, it formed a hexaacetate and a dimethyl ether (with methyl iodide and silver oxide). The UV absorption spectrum was closely similar to that of norswertianolin. There was, however, no change in the UV maxima in ethanolic sodium acetate; the C_3-oxygen function is, therefore, substituted. In ethanolic sodium hydroxide, decomposition of the chromophoric system was observed. These observa-

[3] Whatman No. 50.



Scheme I—Isolation of xanthone-O-glucosides from S. purpurascens

tions, together with the positive quinol test shown by the compound, locate the *p*-hydroxy groups at C_5 and C_8. In the NMR spectrum of the compound, four aromatic protons appeared as *ortho*- and *meta*-doublets; these are associated with C_6-, C_7-, C_2-, and C_4-protons. In addition, there were seven protons due to β-glucoside linkage, three protons associated with an aromatic methoxyl (C_3-OCH_3), and a strongly deshielded proton associated with either C_1- or C_8-OH. The C_2-H in this compound appeared at a significantly lower field (δ 6.52) than the normal position of a 1,3,5,8-tetrahydroxy/methoxy xanthone (δ 6.36) (4). On this basis, the *O*-glucoside linkage appeared to be located at C_1. This conclusion was supported by the fact that the dimethyl ether, on hydrolysis, gave 1-hydroxy-3,5,8-trimethoxyxanthone. Therefore, the compound was identified as 5,8-dihydroxy-3-methoxyxanthone-1-*O*-glucoside (II) (swertianolin).

Swertianolin was previously reported to occur in *S. japonica* (14) and *S. tosaensis* (15), but its structure was only partially defined. The point of glucoside linkage (at C_1 or C_8) and the position of the methyl ether were not settled in earlier studies (14, 15). While the structure of the aglucone moiety was subsequently established (20), this study demonstrates the position of the glucoside linkage as in II.

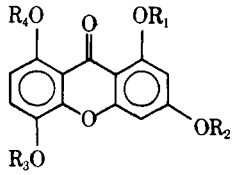
Xanthone-*O*-glucoside (III)—Compound III, $C_{20}H_{20}O_{11}\cdot H_2O$, mp 220–222°, $[\alpha]_D^{25}$ −110 ± 2°, is isomeric with swertianolin. The UV absorption spectrum was also closely similar to that of swertianolin. It formed a hexaacetate and a dimethyl ether (with ethereal diazomethane and formamide). On hydrolysis with dilute sulfuric acid, it furnished 1,5,8-trihydroxy-3-methoxyxanthone and glucose. In its mass spectrum, there was no discernible molecular ion peak but there was a fragment ion peak due to the aglucone moiety at *m/e* 274 (100%) and further fragmentation at *m/e* 245 (18) 244 (7), and 231 (14). The latter fragment ion peaks suggested the presence of C_3-OCH_3 and C_5-OH groups in the xanthone moiety (6, 7, 9). The NMR spectrum of the compound also showed a close similarity with that of swertianolin with only one exception: the C_7-H, instead of the C_2-H, showed a marked downfield shift (by about 0.4 ppm). The NMR spectrum of the acetyl derivative indicated six acetyl groups. Of these, two appeared at a significantly lower field (δ 2.40 and 2.48) than the remaining four (δ 1.85, 1.90, 2.02, and 2.08), suggesting their assignments as aromatic acetoxy. The upfield signals of the remaining acetoxy groups are in good agreement with the chemical shifts of the acetylated β-D-glucopyranoside (21, 22). The signals from the aromatic protons at C_2 and C_4 (δ 6.83 and 6.60, respectively) in the hexaacetate were practically the same as those from the corresponding protons of 1,5,8-triacetoxy-3-methoxyxanthone (bellidifolin triacetate) (δ 6.84 and 6.58) and swerchirin diacetate (δ 6.84 and 6.54) (23). However, the signals from C_6 and C_7 (δ 7.52 and 7.30) differed significantly from the corresponding positions of the last two compounds (δ 7.20 and 6.80 and δ 7.17 and 6.84, respectively).

On this basis, it appeared that the glucoside hexaacetate must bear an acetoxy at C_1 and that the glucoside linkage is at C_8. In consonance with this conclusion, the dimethyl ether, on hydrolysis, afforded 1,3,5-trimethoxy-8-hydroxyxanthone. This is a new tetraoxygenated xanthone, not encountered before in nature or prepared synthetically. Its identity was established from spectral data and from derivatization to 1,3,5,8-tetramethoxyxanthone (4). Therefore, the xanthone-*O*-glucoside was identified as 1,5-dihydroxy-3-methoxyxanthone-8-*O*-glucoside (III), which was named isoswertianolin. This study is the first demonstration of the occurrence of isoswertianolin in nature. This observation seems to be biogenetically significant since this is the first example of the natural occurrence of a xanthone-8-*O*-glucoside.

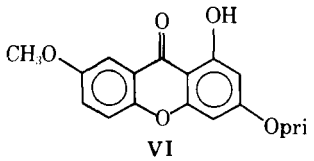
While the natural occurrence of xanthone-*O*-glycosides is restricted so far to the members of the family Gentianaceae (Table I), the related *C*-glucosides—*viz.*, mangiferin (IV) and isomangiferin (V), occur widely among angiosperms (3, 22, 24, 25). Mangiferin has also been reported to occur with its 3-methyl ether (homomangiferin) in Liliaceae (25). The available data in the literature indicate that there could be more xanthone-*O*-glycosides in

Table I—Distribution of Xanthone-*O*-glycosides in Gentianaceae

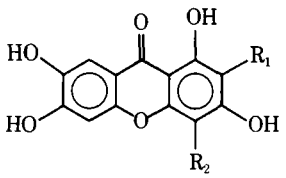
Xanthone	Species	Reference
Gentioside (VI)	*Gentiana lutea*	28
Gentiakochianoside (VII)	*G. kochiana, Swertia decussata*	29
Gentiacauloside (VIII)	*G. kochiana, G. acaulis*	28
Isogentiacauloside (IX)	*G. kochiana*	28
Norswertianolin (I)	*S. randaiensis, S. purpurascens*	10
Swertianolin (II)	*S. japonica, S. tosaensis, S. purpurascens*	14, 15
Isoswertianolin (III)	*S. purpurascens*	
Swerchirin glycoside (X)	*Frasera caroliensis*	30
Xanthone-*O*-glucoside (XI)	*S. bimaculata*	31
Xanthone-*O*-glucoside (XII)	*S. bimaculata*	31
Decussatin primeveroside (XIII)	*G. verna*	32
Corymbiferin-*O*-glycoside (XIV)	*G. corymbifera*	26



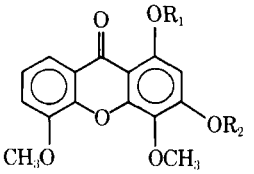
I: R_1 = glu, R_2 = R_3 = R_4 = H
II: R_1 = glu, R_2 = CH_3, R_3 = R_4 = H
III: R_1 = R_3 = H, R_2 = CH_3, R_4 = glu
X: R_1 = gly, R_2 = R_3 = CH_3, R_4 = H



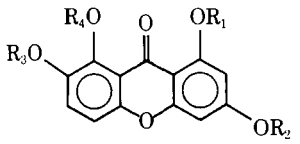
VI



IV: R_1 = glu, R_2 = H
V: R_1 = H, R_2 = glu



XI: R_1 = H, R_2 = glu
XII: R_1 = glu, R_2 = H



VII: R_1 = R_4 = H, R_2 = CH_3, R_3 = pri
VIII: R_1 = H, R_2 = R_4 = CH_3, R_3 = pri
IX: R_1 = R_3 = CH_3, R_2 = pri, R_4 = H
XIII: R_1 = pri, R_2 = R_3 = R_4 = CH_3

glu = β-D-glucosyl
gly = glycosyl (nature of sugar moieties unknown)
pri = primeverosyl

nature than are recorded to date. Polyoxygenated xanthones isolated from gentianaceous plants were often present in a bound (glycoside) form (16, 23, 26). The liberation of these compounds into polyoxygenated xanthones required treatment with dilute acids (16); hydrolysis also takes place during crystallization from protic solvents. When modified isolation procedures can be adopted, it will be interesting to see whether more xanthone-bearing taxa will be found to contain the corresponding *O*-glycosides. The results from such investigations would suggest biochemical significance of this blocking (glycosidation), which appears to be characteristic of the members of the Gentianaceae.

The study of xanthone-*O*-glycosides could also be therapeutically significant since a number of such compounds produced remarkable pharmacological and other biological activities (11, 27). The total xanthone-*O*-glucosides of *Canscora decussata* produced marked antipsychotic effects, while the corresponding aglucones and the *C*-glucoside, mangiferin, showed monoamine oxidase inhibitor activities (5, 9, 27). The therapeutic properties of *C. decussata* plant extracts (1, 12) could, therefore, be ascribed to the contained xanthone-*O*-glucosides. Mangiferin, constituting about 80% of the total xanthones of this plant, acts as a potentiator of the activities of the xanthone-*O*-glucosides.

Selected pharmacological screening of the total xanthone-*O*-glucosides (I–III), in doses of 25–50 mg/kg, produced definite signs of central nervous system (CNS) depression in albino mice and rats. The effect was manifested by an initial transient hyperactivity (with flag-tail in the case of albino mice) followed by moderate to deep depression, intact reflexes and response to external stimuli, potentiation (165%) of hexobarbital hypnosis in albino mice, and complete protection against amphetamine (20 mg/kg) and 5-methoxy-*N*,*N*-dimethyltryptamine (10 mg/kg) induced (33) toxicities. The total *O*-glucosides markedly potentiated (120%) the analgesic activity of subanalgesic doses (2 mg/kg ip) of morphine but had no analgesic activity *per se*. In higher doses (100 mg/kg ip) only, the total xanthone-*O*-glucosides offered protection to electroshock seizure (50%) and pentylenetetrazol- (70 mg/kg sc) induced convulsions (60%). Additionally, the total *O*-glucosides showed cardiovascular stimulant (in dose of 1 mg) and choleretic activities. These activities were determined according to methods reported earlier (5). The xanthone aglucones, on the other hand, produced dose-dependent weak CNS stimulant activities. The total xanthone-*O*-glucosides were nontoxic up to 200 mg/kg ip. These observations seem to indicate that the curative properties ascribed to *S. purpurascens* plant extracts (1) are primarily due to the contained xanthone-*O*-glucosides.

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PHARMACEUTICAL ANALYSIS

Quantitative TLC of Salicylazosulfapyridine

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Abstract □ Nine lots of raw material salicylazosulfapyridine from six suppliers were analyzed by an early spectrophotometric method. The lower limit of purity set by this method is 88%. Using a highly purified reference standard, results of the spectrophotometric method were compared to a salicylazosulfapyridine-specific, quantitative TLC method. Results confirmed the nonspecificity of the spectrophotometric method. In an extreme case, material with a 94% spectrophotometric purity value was shown to be only 85% by quantitative TLC. As many as eight extraneous spots in addition to salicylazosulfapyridine were found in some lots of raw material when developed chromatographs were sprayed with an azo-indicating stain. The minimum visible detection level of the impurities was between 0.01 and 0.1% of the total salicylazosulfapyridine spotted. Isolated impurities showed spectrophotometric absorbance at the wavelength of the spectrophotometric method, resulting in erroneous purity values. Drug recovery by the quantitative TLC method was 98% with an assay standard deviation of less than ±0.5%. These results led to commercial upgrading of synthesis and purification, so that raw material salicylazosulfapyridine at the 96% level by quantitative TLC was subsequently available for drug production.

Keyphrases □ Salicylazosulfapyridine—quantitative TLC analysis, compared to literature spectrophotometric method □ TLC—analysis, salicylazosulfapyridine, compared to literature spectrophotometric method

Salicylazosulfapyridine (I), indicated for use in the treatment of ulcerative colitis, has been marketed in the United States for about 20 years. The first monograph for this drug appeared in 1953 (1). Chemical specifications for I found in that reference are as current as can be found and call for a purity of not less than 88%.

During the development of I dosage forms in this laboratory, it was necessary to explore analytical procedures for assurance of raw material purity as well as chemical stability. Three methods have been described: a titanium trichloride titration used in 1925 (2), a spectrophotometric procedure (2), and the more recent polarographic technique (3). Both the spectrophotometric and the azo titration procedures were included in Ref. 1. These methods were compared by Berggren and Hansen (2), who theorized that the similarity of potency values obtained for I raw material was due to the specificity of both assays for the azo linkage (—N=N—). More recent studies (3) showed that normal production lots of I contain azo impurities which have been proven to be spectrally active. Thus, neither method can be considered specific or meaningful for I potency unless azo impurities are first removed.

Consideration has been given to polarography (3) in an attempt to overcome the impurity disturbances of the spectral method; however, in the final analysis of this work, an average difference of only 0.6% between the two methods is found. This slight difference indicates that polarography is not a significantly more specific assay than spectrophotometry for production lots of I. It was further stated that the spectrophotometric method was unquestionably more sensitive and rapid. Therefore, it was evident from the literature reviewed that a specific, quantitative assay for I did not exist.

Kiger and Kiger (4) reported qualitative TLC techniques for I in their work on sulfonamide differentiation. Two distinct spots for this drug were reported, and one was designated as an impurity. After repeating the qualitative TLC procedure in these laboratories, it was confirmed that indeed two spots were visible with the naked eye at the reported R_f