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XANTHONE AND FLAVONOL CONSTITUENTS OF *SWERTIA HOOKERI**

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Key Word Index—*Swertia hookeri*; Gentianaceae; 1-*O*-stearyl-3,5-dimethoxyxanthone; 3-*O*-stearyl-3′,4′,5,7-tetra-*O*-methylquercetin; 1-hydroxy-3,5-dimethoxyxanthone; tetraoxygenated xanthones; 8-*O*-β-D-glucosyl-1,3-dihydroxy-5-methoxyxanthones; 8-*O*-β-D-glucosyl-1,5-dihydroxy-3-methoxyxanthone.

Abstract—The whole plant of *Swertia hookeri*, collected at flowering has been shown to contain two tri- and nine tetraoxygenated free, glucosyloxy, and stearyl ester xanthones and one flavonol stearyl ester. Among these, three are previously unreported in nature and one was known previously only as a synthetic compound. The xanthones are based on 1,3,5,-, 1,3,5,8- and 1,3,7,8-oxygenated systems with the middle oxygenation pattern predominating. The two ester compounds appeared only at the flowering stage. Plants collected at the pre-flowering stage gave the corresponding free compounds. The biochemical and biological significance of these findings are appraised.

INTRODUCTION

Swertia hookeri C. B. Clarke (Gentianaceae), native to the mountains of the north-eastern Himalayas (altitude 14 000–17 000′), is a tall flowering species which differs considerably in length, shape and colour of roots (deep yellow) from the smaller members of this genus [2]. The extract of the plant is used by people of the north-eastern Hill region for a variety of therapeutic purposes, e.g. in the treatment of microbial infections in man, in hypertension, and as a mood elevator. Phytochemical investigation of this species has not previously been carried out. The present investigation of the whole plant, collected at flowering, resulted in the isolation and characterization of a number of free and substituted xanthone and flavonol constituents.

RESULTS AND DISCUSSION

Extensive column and layer chromatography of the petrol and EtOH extracts of the whole plant afforded a xanthone (**1**) and a flavonol (**2**) fatty acid ester together with eight free (**3–10**) and two glucosyloxy (**11** and **12**) xanthones. Among these, nine were previously encountered in other *Swertia* species or known as synthetic compounds. These are 1-hydroxy-3,5-dimethoxy- (**3**) [3], 1,3,5,8-tetrahydroxy- (**4**) [4, 5], 1,3,8,-trihydroxy-5-methoxy- (**5**) [4], 1,5,8-trihydroxy-3-methoxy- (**6**) [4], 1-hydroxy-3,5,8-trimethoxy (**7**) [4], 1,3,5,8-tetramethoxy- (**8**) [4], 1,3,7,8-tetrahydroxy- (**9**) [5], 1-hydroxy-3,7,8-trimethoxy- (**10**) [5], and 8-*O*-β-D-glucosyl-1,5-dihydroxy-3-methoxyxanthone (**11**) [6, 7]. The characterization of only the three new compounds (**1**, **2** and **12**) is described here.

Compound 1

This compound, mp 177–180°, $C_{33}H_{46}O_6$, showed UV maxima in MeOH typical of 1,3,5-trioxygenated xanthones [3]. The UV maxima remained unaltered in the presence of the usual shift reagents. The compound was recovered unchanged after treatment with MeI–NaH in tetrahydrofuran at room temperature. It did not exhibit a molecular ion peak in its MS but significant fragment ion peaks appeared at *m/e* 284 and 272 due to the moieties of a xanthone fatty acid ester. On hydrolysis with aqueous HCl it afforded 1-hydroxy-3,5-dimethoxyxanthone and stearic acid. These data suggest 1-*O*-stearyl-3,5-dimethoxyxanthone (**1**) as its structure.

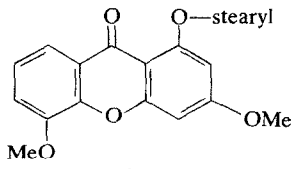
Compound 2

This compound, $C_{37}H_{52}O_8$, showed UV maxima typical of a peralkylated quercetin derivative [8]. The maxima remained unaltered in the presence of NaOAc. However, slow decomposition was observed in the presence of both NaOMe and $AlCl_3$. It did not exhibit a molecular ion peak in its MS but significant fragment ion peaks appeared at *m/e* 358 and 284 due to the moieties of a flavonol fatty acid ester. On prolonged standing, even in neutral solution, it was converted into 3′,4′5,7-tetra-*O*-methylquercetin and stearic acid. These data suggest 3-*O*-stearyl-3′,4′,5,7-tetra-*O*-methylquercetin (**2**) as its structure.

* Part 27 in the series: 'Chemical Constituents of Gentianaceae'. For Part 26 see [1].

Compound **12**

This compound, mp 205–210°, $C_{20}H_{20}O_{11}\cdot H_2O$, was a dihydroxymonomethoxyxanthone-*O*-glycoside as evidenced from its MS and ^1H NMR spectra and formation of an hexaacetate. It showed UV maxima characteristic of 1,3,5,8-tetraoxygenated xanthones [4]. The maxima shifted bathochromically in the presence of both $AlCl_3$ and NaOAc, thereby suggesting free OH groups, respectively, *peri* (C_1 or C_8) and *para* (C_3) to the carbonyl group. The spectrum remained unchanged in MeOH–NaOH thereby precluding a *p*-di-OH function in the molecule. On hydrolysis with emulsin it gave glucose and 1,3,8-trihydroxy-5-methoxyxanthone. The dimethyl ether of the glucoside, prepared by treatment with Me_2SO_4–K_2CO_3, on acid hydrolysis gave 1,3,5-trimethoxy-8-hydroxyxanthone [6]. These data suggest 1,3-dihydroxy-5-methoxyxanthone-8-*O*-β-D-glucoside (**12**) as its structure.



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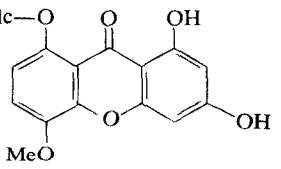

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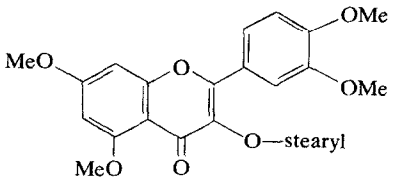

2

This is the first report of a 1,3,5-trioxygenated xanthone and, also, of xanthone and flavonol fatty acid esters in a *Swertia* species. Curiously, 1,3,5- and 1,3,7-trioxygenated xanthones [9, 10] are extremely rare in members of the genera *Swertia* and *Gentiana* which liberally elaborate 1,3,5,8-/1,3,7,8-tetra and 1,3,4,5,8-/1,3,4,7,8-pentaoxygenated xanthones [2]. Further, the relative abundance of the two tetraoxygenated patterns (1,3,5,8-/1,3,7,8-) of xanthones in *Swertia* was found to be inversely proportional, the latter pattern in most species being predominant [4, 11]. In the present investigation with *S. hookeri*, however, 1,3,5,8-tetraoxygenated xanthones were obtained in very high yield (*ca* 20% of dry weight of the plant). This observation is important from the medicinal point of view. Pharmacological screening of the free, glucosylated, and stearyl ester xanthones of *S. hookeri* and of other *Swertia* species produced varying responses on the central nervous and cardiovascular systems in laboratory animals [12–14]. Some of these actions were consistent with the therapeutic properties of *S. hookeri* plant extract.

EXPERIMENTAL

The general descriptions are as reported in a recent paper [15]. Plant material was collected twice (June and July 1975) from the Sela top, District Kameng, Arunachal Pradesh, India. A voucher specimen of each has been preserved at the Pharmaceutical Chemistry Research Laboratory, Department of Pharmaceutics, Banaras Hindu University, Varanasi-5.

Extraction of S. hookeri. Dried and milled whole plant (80 g) was continuously extracted in a Soxhlet with petrol (60–80°) and then EtOH (40 hr. each). The 2 extracts were separately processed.

Treatment of the petrol extract. The petrol extract was conc (250 ml) and kept overnight at room temp. when a brownish-yellow solid (fraction A, 1.6 g) separated. The solid was collected by filtration and the petrol mother liquor was evapd to give a brown gummy material (fraction B, 2.2 g).

Separation of xanthones from fraction A. A portion of the solid (80 mg) was dissolved in $CHCl_3$ and the soln was extracted with aq. Na_2CO_3 (2%, 100 ml).

1,3,8-*Trihydroxy-5-methoxyxanthone* (**5**). The aq. Na_2CO_3 soln, on acidification followed by extraction with $CHCl_3$, afforded a residue (21 mg) which crystallized from EtOH as yellow needles, mp 261–263°. The mp, mmp, UV, and R_fs were identical with those of 1,3,8-trihydroxy-5-methoxyxanthone [4].

1,5,8-*Trihydroxy-3-methoxyxanthone* (**6**). The $CHCl_3$ layer, after usual work-up, afforded 1,5,8-trihydroxy-3-methoxyxanthone as yellow crystals (52 mg), mp 270–272°. The identity of the cpd was established by direct comparison (mp, mmp, co-TLC, UV) with an authentic sample [4].

Separation of xanthones from fraction B. A portion of the gummy material (222 mg) was dissolved in Et_2O and processed for neutral, phenolic and carboxylic consitituents in the usual fashion.

1-*O*-*Stearyl*-3,5-*dimethoxyxanthone* (**1**). During the separation of phenolic and carboxylic constituents, a solid appeared at the Et_2O-aq. alkali interface and was filtered, washed, dried and crystallized from MeOH–Me_2CO as pale yellow flakes (12 mg), mp 177–180°. UV λ_{max} nm (log ε): 245 (4.24), 280 sh (3.60), 300 sh (3.63). MS (rel. int., %): *m/e* 284. (30), 272 (20), 257 (72), 243 (12), 241 (100). (Found: C, 73.1; H, 8.8. $C_{33}H_{46}O_6$ requires: C, 73.6; H, 8.5%). Hydrolysis with aq. HCl (6%) on a steam bath gave 1-hydroxy-3,5-dimethoxyxanthone, mp 170–171° (mp, mmp, co-TLC, UV) [2, 3] and stearic acid (co-TLC, MS). The phenolic fraction (fraction C) was obtained as a greenish-yellow solid (23 mg). The neutral fraction was chromatographed over a column of Si gel (18×2 cm) using petrol (500 ml), C_6H_6 (1 l.), and C_6H_6–$CHCl_3$ (1:1, 1 l.) as eluents. Fractions (250 ml) were collected and monitored by TLC.

1,3,5,8-*Tetramethoxyxanthone* (**8**). The C_6H_6 eluates were combined and subjected to PLC (C_6H_6–HOAc, 50:1). The upper blue fluorescent zone in short wave UV, $R_f \sim 0.7$, was eluted with $CHCl_3$ and crystallized from EtOH as colourless needles (18 mg), mp 224–225°. The mp, mmp, UV and R_fs were identical with those of 1,3,5,8-tetramethoxyxanthone [4]. Treatment of **6** with Me_2SO_4–K_2CO_3 in Me_2CO under reflux (42 hr) and usual work-up also afforded this compound. This is the first report of the natural occurrence of **8**, previously known only as a synthetic compound [4].

1-*Hydroxy*-3,5-*dimethoxyxanthone* (**3**). The yellow lower PLC zone, $R_f \sim 0.5$, was eluted with $CHCl_3$ and crystallized from EtOH as yellow needles (15 mg), mp 172°. The mp, mmp, UV and R_fs were identical with those of 1-hydroxy-3,5-dimethoxyxanthone [3].

Separation of xanthones from fraction C. This fraction on TLC showed one major yellow and a number of I_2-positive spots due to triterpenoid constituents. The latter components responded to Liebermann–Burchardt reagent for triterpenoids. Separation of the yellow cpd was accomplished by PLC.

1-*Hydroxy*-3,7,8-*trimethoxyxanthone* (**10**). The yellow PLC zone, $R_f \sim 0.3$ (C_6H_6–HOAc, 98:2), was processed in the usual fashion to afford **10** as yellow needles (11 mg). The identity of the xanthone was confirmed by direct comparison (mp, mmp, UV, co-TLC) with an authentic sample [2, 4].

Treatment of the EtOH *extract.* The EtOH extract on standing overnight at room temp. afforded a deep brown solid (172 mg) separated by filtration. The mother liquor was concd under red. pres., poured into aq. HOAc (4%, 200 ml) and kept overnight when a brown solid was separated. The solid (fraction D, 14.5 g) was collected and the conc clarified acidic aq. soln extracted with Et_2O (10×250 ml). The combined Et_2O extract was worked up in the usual fashion to give a dull yellow solid (fraction E, 282 mg). The aq. layer was further extracted with EtOAc (5×250 ml). The combined EtOAc extract after usual processing gave another dull yellow solid (fraction F, 314 mg) consisting of strongly polar xanthones.

Separation of xanthones from fraction D. A portion of the solid (1.4 g) was triturated successively with hot C_6H_6 (6× 50 ml), $CHCl_3$ (10×50 ml) and MeOH (50 ml). A further sample of xanthone **6** (0.8 g) was obtained as MeOH-sparingly sol. solid. TLC of the MeOH mother liquor showed the presence of 1,3,5,8- (major) and 1,3,7,8- (minor) tetrahydroxyxanthones.

1,3,5,8-*Tetrahydroxyxanthone* (**4**). The MeOH mother liquor was concd and chromatographed over a Si gel column (40×2.5 cm) using $CHCl_3$, MeOH and various $CHCl_3$/MeOH mixtures. The early $CHCl_3$–MeOH (19:1) eluates gave 1,3,5,8-tetrahydroxyxanthone as a greenish-yellow solid (180 mg), mp 295–298°. The identity of the cpd was established by direct comparison (mp, mmp, UV, co-TLC) with an authentic sample [4].

1,3,7,8-*Tetrahydroxyxanthone* (**9**). The combined MeOH washings from the Si gel column on evapn gave a dull yellow residue which crystallized from EtOH to give 1,3,7,8-tetrahydroxyxanthone as pale yellow microcrystals (12 mg), mp 330–333° (mp, mmp, co-TLC) [4]. From the hot C_6H_6- and $CHCl_3$-soluble fractions, further quantities of xanthone **5** (10 mg) and **6** (22 mg) were obtained.

Separation of constituents from fraction E. The dull yellow solid was triturated with hot petrol (100 ml), C_6H_6 (100 ml) and $CHCl_3$ (100 ml). The $CHCl_3$-insol. solid (218 mg) was a complex mixture of phenolic glycosides. The petrol- and C_6H_6-soluble fractions were combined (as their TLC behaviour was similar), concd and subjected to PLC (C_6H_6–HOAc, 19:1).

1-*Hydroxy*-3,5,8-*trimethoxyxanthone* (**7**). The upper yellow PLC zone, $R_f \sim 0.6$, after usual work-up gave 1-hydroxy-3,5,8-trimethoxyxanthone as light yellow needles (11 mg), mp, 205–207° (mp, mmp, UV, co-TLC) [4].

3-*O*-*Stearyl*-3′,4′,5,7-*tetra*-*O*-*methylquercetin* (**2**). The lower olive-green UV-fluorescent zone, R_f 0.2, was eluted with $CHCl_3$–MeOH. Evapn of the solvent gave a light yellow semi-solid (7 mg). UV λ_{max} nm: 250, ~280, 362. MS (rel. int., %): *m/e* 358 (100), 357 (3), 343 (12), 284 (68). (Found: C, 70.8; H, 8.0. $C_{37}H_{52}O_8$ requires: C, 71.1; H, 8.3%). Hydrolysis of **2** with aq. HCl (4%, at ordinary temp.) followed by usual work-up gave 3′,4′,5,7-tetra-*O*-methylquercetin as a pale yellow solid, mp and mmp 145–148°. UV λ_{max} nm: 252, 270–272, 365; $\lambda_{max}^{MeOH-AlCl_3}$: 265, ~300, 422–425; *m/e* 358 (M^+); and stearic acid (co-TLC, MS).

Separation of glucosyloxyxanthones from fraction F. The solid was triturated with hot $CHCl_3$ (100 ml). The $CHCl_3$-insol. solid (115 mg) showed two major components, R_f 0.65 (major), 0.5 (major), and several minor components, R_f 0.1–0.8, on analytical TLC (*n*-BuOH–HOAc–H_2O, 4:1:2). Trituration with hot Me_2CO removed the less polar major component and all the minor constituents.

8-*O*-β-D-*Glucosyl*-1,3-*dihydroxy*-5-*methoxyxanthrone* (**12**). The Me_2CO-insol. solid crystallized from MeOH as snuff-coloured crystals (66 mg), mp 205–210°; R_f 0.52; $[\alpha]_D^{28} - 102°$ (*c* 0.24, MeOH). UV λ_{max} nm (log ε): 250 (4.55), 265 sh (4.27), 275 (4.25), 329 (4.15), 375 (3.70). ^{1}H NMR (DMSO-d_6): δ 12.98 (1H, *s*, exchangeable with D_2O, C_1-OH), 7.18 (1H, *d*, *J* = 9.5 Hz, H-6), 7.08 (1H, *d*, *J* = 9.5 Hz, H-7), 6.53 (1H, *d*, *J* = 3 Hz, H-4), 6.32 (1H, *d*, *J* = 3 Hz, H-2), 5.03 (1H, *m*, glucosyl anomeric proton), 3.86 (3H, *s*, OMe). (Found: C, 52.7; H, 4.3. $C_{20}H_{20}O_{11}\cdot H_2O$ requires: C, 52.9; H, 4.0). The hexaacetate, prepared by warming the glucoside (15 mg) with Ac_2O (2 ml) and C_5H_5N (0.1 ml), was obtained as an amorphous powder (14 mg); *m/e* 688 (M^+, 1.2%), 358 (95), 357 (14), 331 (98), 316 (38), 274 (100), 273 (5), 259 (4), 245 (28). Hydrolysis of the glucoside (10 mg) with emulsin, according to a published method [11], gave 1,3,8-trihydroxy-5-methoxyxanthone [4] and glucose (PPC). The dimethyl ether was prepared by dissolving the glucoside (9 mg) in DMF (1 ml) and MeOH (10 ml) and treating the soln with Et_2O–CH_2N_2. Work-up in the usual fashion gave a brown gummy material which showed two spots, R_f 0.45 (major) and 0.7 (minor) on TLC ($CHCl_3$–HOAc, 19:1). These were separated by PLC. Hydrolysis of the major component with aq. HCl afforded 1,3,5-trimethoxy-8-hydroxyxanthone, mp 210–212° (mmp, co-TLC, UV) [6, 11] and glucose (PPC).

8-*O*-β-D-*Glucosyl*-1,5-*dihydroxy*-3-*methoxyxanthone* (**11**). The Me_2CO-sol. portion (fraction F), on concn gave a pale yellow solid (24 mg), mp and mmp 222–225°. Hydrolysis with emulsin gave 1,5,8-trihydroxy-3-methoxyxanthone [6] and glucose (PPC). The glucoside, in tetrahydrofuran, was permethylated with MeI and NaH. The permethyl ether was hydrolysed with aq. HCl to give 1,3,5-trimethoxy-8-hydroxyxanthone [6, 11] and 2,3,4,6-tetra-*O*-methylglucose (PPC, ^{1}H NMR).

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