A New Penta-oxygenated Xanthone from the Fern *Cystopteris fragilis*

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Cystopteris fragilis, Pteridophyta, Aspleniaceae, 1,6-Dihydroxy-3,5,7-trimethoxyxanthone

A new penta-oxygenated xanthone has been isolated from aerial parts of the fern *Cystopteris fragilis*. By spectroscopic and chemical methods this compound has been shown to be 1,6-dihydroxy-3,5,7-trimethoxyxanthone which is of biosynthetic interest in ferns.

Introduction

Pharmacological activity (*e.g.* antipsychotic action [1]) and chemotaxonomic interest of xanthones may explain the increasing interest in these substances in recent years. It has been suggested [2] that *Cystopteris fragilis* complex is the most interesting biosystematic problem in ferns. Xanthone analysis may be of interest in the study of relationships of the taxa of this complex since previous work on the xanthones and flavonoids of Appalachian *Asplenium* complex [3] and *Asplenium adiantum-nigrum* complex [4] has shown that these two groups of ferns provide a classic example of additive inheritance of chemical characters.

Earlier work on the chemical constituents of *Cystopteris fragilis* has led to the identification of two C-glycosylxanthones (mangiferin and isomangiferin) [5] and four flavonoids based on kaempferol (the 3-O-glucoside, the 3,4'-bis-glucoside, the 3-O-(3''-sulphate)glucoside and the 3-O-(6''-sulphate)glucoside [6]. In the present work a penta-oxygenated xanthone has been found for the first time in ferns and it has been shown that this compound (isolated from *Cystopteris fragilis* Bernh) is 1,6-dihydroxy-3,5,7-trimethoxyxanthone (**1**) which is a new natural product of biosynthetic interest in ferns.

Materials and Methods

Air dried aerial parts (50 g) of *Cystopteris fragilis* Bernh (collected on volcano Etna, Sicily) were homogenized and extracted 3 times with hot 95% ethanol; evaporation of the solvent *in vacuo* gave 14 g of residue. The new xanthone (**1**) was isolated

by preparative paper chromatography on Whatman 3MM paper in *n*-butanol-acetic acid-water (4:1:5, upper phase) (BAW). The bands, as observed under ultraviolet light with ammonia vapor (colour reactions: yellow to orange in UV+NH₃) were cut out, eluted with ethanol, concentrated *in vacuo* and rechromatographed in 15% acetic acid (HOAc) and in *n*-butanol-ethanol-water (4:1:2.2) (BEW). Further purification was carried out on a silica column chromatography (eluting with benzene) which gave 6 mg of xanthone **1**. *R_f* values on Whatman N.1 paper are: 0.79 in BAW; 0.81 in BEW; 0.17 in 15% HOAc. *R_f* value on TLC on silica: 0.73 in benzene-methanol (4:1). UV, MS and ¹H NMR data of xanthone **1** are shown in Table I.

Complete demethylation of xanthone (**1**)

The xanthone (2 mg) and HI (4 ml; *d* = 1.7) were refluxed for 6 h in the dark. Removal of hydriodic acid under high vacuum gave a residue which was chromatographed. The single spot in TLC on silica (solvent system: benzene-methanol, 4:1) and on paper chromatography (solvent system: AcOH–37% HCl–water, 30:3:10) was indistinguishable from a sample of 1,3,5,6,7-penta-hydroxyxanthone from *Cascora decussata*.

Results and Discussion

Colour reactions and UV spectral analysis in the presence of the customary shift reagents (Table I) suggest that the isolated compound is a xanthone with a free hydroxyl group at position 3 and/or 6 (shift with NaOAc) and a free hydroxyl group at position 1 and/or 8 (shift with AlCl₃ and AlCl₃/HCl) [7]. The mass spectrum of this compound (Table I) exhibited a molecular ion at *m/z* 318 for C₁₆H₁₄O₇ in accord with a xanthone containing two hydroxyl groups and three methoxyl groups. The ¹H NMR spectrum (Table I) confirmed the presence of these substituents since signals were observed for three methoxyl groups (δ 3.89, 3.97 and 4.01) and for two hydroxyl groups (δ 12.95 and 5.08) one of which must be in position 1 or 8 [8]. In addition the position and coupling of two *meta*-split doublets (*J* = 2.3 Hz) at δ 6.52 and 6.36 suggested [8] 1,3-dioxygenation in A-ring of the xanthone. A singlet at δ 7.42 was assigned to C-8 proton in spite of the fact that a C-8 proton would give a signal at lower field (between 7.70 and 8.05 [9]) since the signal of this proton is shifted to higher field by the presence of three substituents on the B-ring of the isolated xanthone [8]. This conclusion is confirmed by the ¹H NMR spectrum of synthetic 1,3,5,6,7-pentamethoxyxanthone [10] which

Table I. UV, MS and ^1H NMR data of xanthone **1**.

UV $\lambda_{\text{max}}^{\text{MeOH}}$ (nm)	MS m/z (rel. int.)	^1H NMR ($\delta(\text{CDCl}_3)$, 250 MHz)
–	352, 315, 255	318 (M^+ , 100%)
AlCl_3	397, 345, 268	303(8)
AlCl_3/HCl	396, 330, 265	288(12)
NaOAc	377, 260	275(10)
NaOMe	377, 261	257(11)
		245(3)
		229(4)
		145(7)
		123(5)

shows a singlet at $\delta(\text{CDCl}_3)$ 7.38 (C-8 proton); in addition calculations based on the work of Barraclough *et al.* [8] suggest that 8-H of a xanthone with three hydroxy- and/or methoxy-substituents on the B-ring should appear as singlet a $\delta(\text{CDCl}_3)$ 7.13 whereas 7-H, 6-H and 5-H of such xanthone are predicted to give a singlet at $\delta(\text{CDCl}_3)$ 6.10, 6.46 and 6.25 respectively. The presence of a C-8 proton in the B-ring of the isolated xanthone is confirmed by the absence in the mass spectrum (Table I) of peaks from the loss of OH, H_2O and CHO from the molecular ion since these peaks are typical [11] for xanthenes and related compounds with a methoxyl substituent *peri* to carbonyl group.

The above observations show that the isolated xanthone must be 1,3-dihydroxy-5,6,7-trimethoxyxanthone or 1,6-dihydroxy-3,5,7-trimethoxyxanthone. The presence of a 1,3,5,6,7-oxygenation pattern in the isolated xanthone was confirmed by complete demethylation of this compound with HI which gave 1,3,5,6,7-pentahydroxyxanthone identified by direct comparison with an authentic sample from *Cascora decussata* [12]. Since the UV spectra of the isolated xanthone in the presence of NaOAc and NaOMe are superimposable (Table I), the isolated xanthone seems to be 1,6-dihydroxy-3,5,7-trimethoxyxanthone

since the above spectra are not superimposable [7] if a 1,3-dihydroxyl system is present in the xanthone. This conclusion was confirmed by direct comparison of the isolated xanthone (**1**) with an authentic sample of 1,6-dihydroxy-3,5,7-trimethoxyxanthone which was obtained by selective demethylation [13] of 1,3,5,6,7-pentamethoxyxanthone prepared according to Ghosal *et al.* [12].

Xanthone **1** is the first penta-oxygenated xanthone isolated from ferns. In 1969 Carpenter *et al.* [14] predicted that 1,3,5,6,7-oxygenated xanthenes should occur in plants if the phenol oxidative coupling biosynthetic mechanism is operative. This suggestion was confirmed in 1974 by Ghosal *et al.* which isolated [12] for the first time a number of xanthenes having the predicted oxygenation pattern from *Cascora decussata* Schult. In the present work it is shown that a xanthone with the above oxygenation pattern is present also in ferns.

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