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SHORT COMMUNICATION

THE STRUCTURE OF EXOSTEMIN, A NEW 4-PHENYL COUMARIN ISOLATED FROM *EXOSTEMMA CARIBAEUM*

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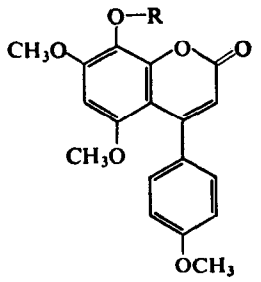
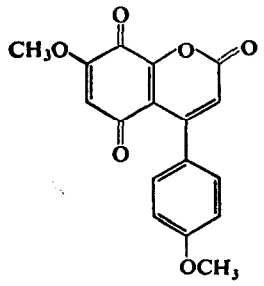
Abstract—The structure of exostemin, a constituent isolated from the benzene extract of the Mexican plant *Exostemma caribaeum* (Rubiaceae), has been established as 8-hydroxy-5,7-dimethoxy-4-(*p*-methoxyphenyl) coumarin (I). This compound is of interest since it is the second B-ring substituted 4-phenylcoumarin to be isolated from a natural source.

IN A PRELIMINARY report[1] we described the isolation and chemistry of exostemin, a new 4-phenylcoumarin for which we assigned the tentative structure (I). This constitution is entirely confirmed in the present communication.

Exostemin (I), $C_{18}H_{16}O_6$, affords an i.r. spectrum ($CHCl_3$ solution) with bands at 3650 cm^{-1} (free hydroxyl group) and 1720 cm^{-1} (α-pyrone[2] C=O). The NMR spectrum shows 3-proton peaks at δ 3·43, 3·85 and 3·97 ppm (methoxyl groups), the significant displacement of the first indicating that one methoxy group (at C_5) is shielded by the aryl substituent. The hydroxyl group gives a signal at 5·51 ppm, and there is a singlet at 6·00 ppm due to the α-vinylic hydrogen of a conjugated carbonyl system, as in a cyclohexenone[3] or in a coumarin.[4] A singlet at 6·35 ppm. corresponds to the isolated aromatic hydrogen between the two methoxy groups. Finally there is a quartet centred at 7·06 ppm (4 H), appropriate to the protons of a *p*-phenylene ring.



Exostemin (I) R = H
(II) R = CH_3CO
(III) R = CH_3



(IV)

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[4] Y. YUKAWA, *Handbook of Organic Structural Analysis*, p. 481, W. A. Benjamin, New York (1965).

An ethanolic solution of exostemin gives no color with $FeCl_3$, but it is phenolic since subsequent addition of $K_3Fe(CN)_6$ solution[5] produced an intense dark-blue colour.

Acetylation (Ac_2O–pyridine method) yields a monoacetate, $C_{20}H_{18}O_7$, (II), which exhibited i.r. carbonyl bands at 1770 (phenolic ester) and 1735 cm^{-1}. The NMR spectrum shows a sharp signal at 2·37 ppm (3 H) due to the acetyl group, and OMe peaks at 3·48, 3·83 and 3·88 ppm The remainder of the spectrum is similar to that of exostemin. That the aromatic hydrogen is not vicinal to the hydroxyl group is inferred from the absence of interaction between the aromatic hydrogen and the acetate group. Methylation with dimethyl sulfate or ethereal diazomethane affords a tetramethoxy compound, $C_{19}H_{18}O_6$ (III), devoid of hydroxyl absorption. The NMR spectral data indicate the presence of four methoxy groups (peaks at 3·48, 3·86, 3·93 and 3·96 ppm). The existence of an ester group in the exostemin molecule, suggested by the i.r. band at 1720 cm^{-1}, was confirmed chemically. *O*-Methylexostemin gave violet color (positive reaction) in the ferric hydroxamate test for esters.[6, 7]

Chromic acid oxidation of exostemin (I) affords a red quinone, $C_{17}H_{12}O_6$ (IV), which gives a positive Craven test[8] and has i.r. bands at 1658 and 1635 cm^{-1} (cf. Ref. 9). The absence of the i.r. hydroxyl band ($CHCl_3$ solution) confirms this group was oxidized during quinone formation, along with the shielded methoxyl group at C_5 now absent from the NMR spectrum. The two remaining OMe groups give rise to peaks at 3·85 and 3·90 ppm. The red color of the quinone suggested it could be an *ortho*-quinone, but it gave a negative reaction with guanidine carbonate[10] and must therefore be a *p*-quinone.

These facts support formula I for exostemin, which then proved to be identical (i.r. and NMR) with 8-hydroxy-5,7-dimethoxy-4(*p*-methoxyphenyl)coumarin synthesized by Mukerjee *et al.*[11] Exostemin is the second example of a coumarin with a substituted 4-phenyl ring. The first example is melannein,[12] isolated from *Dalbergia baroni*. Two other related coumarins are dalbergin and *O*-methyldalbergin.[13]

EXPERIMENTAL

Isolation of Exostemin (I)

The bark and the heartwood of *Exostemma caribaeum* (commonly known as Quina de Michoacán and Copalchi de Jojutla) were pulverized. A batch (450 g) of the finely ground plant was extracted with hexane (4 l.) in a Soxhlet for 12 hr. This extract contained only waxy material and was discarded. Subsequent extraction with benzene gave an extract (3·5 l.) which was concentrated under reduced pressure to a volume of 100 ml and diluted with ether. A yellow solid crystallized (3 g) as prisms, m.p. 157–160° (softens at 120°). TLC revealed the presence of two components which were separated by the same method on a preparative scale, using silica gel G and benzene–methanol (80–20). Iodine was used for visualization. The material with the highest R_f was separated and extracted with ethyl acetate. Exostemin crystallized from ethyl acetate–benzene, m.p. 173–174°; in one experiment, it melted at 193° (identical spectroscopy). The product gave red-orange color with conc. H_2SO_4. ν_{max} ($CHCl_3$) 3650 and 1720 cm^{-1}. (Found: C, 66·0; H, 5·1; O, 29·2; mol. wt., Rast, 319. $C_{18}H_{16}O_6$ required: C, 65·9; H, 4·9; O, 29·2; mol. wt., 328.)

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Exostemin Acetate (II)

Crystallized from ethanol and melted at 203–204°. ν_{max} (KBr) 1770 and 1735 cm^{-1}. NMR peaks at δ 3·48, 3·83 and 3·88 ppm (OMe groups). (Found: C, 64·7; H, 5·2; O, 30·2; mol. wt., 355. $C_{20}H_{18}O_7$ required: C, 64·9; H, 4·9; O, 30·2; mol. wt., 370.)

O-*Methylexostemin (III)*

Crystallized from methanol as a white product (280 mg), m.p. 144–145°. ν_{max} ($CHCl_3$) 1720 cm^{-1}. NMR peaks at δ 3·48, 3·86, 3·93 and 3·96 ppm (Found: C, 66·8; H, 5·4; O, 28·2. $C_{19}H_{18}O_6$ required: C, 66·7; H, 5·3; O, 28·0.)

Chromic Acid Oxidation of Exostemin (I) to (p-*Benzoquinone*) *(IV)*

To a soln. of exostemin (500 mg) in glacial AcOH (5 ml), cooled in an ice–water–salt mixture, a cold soln. of CrO_3 (1 g) in water (1 ml) was added dropwise. After a few minutes the mixture was diluted with ice-water. The resultant red quinone was collected and crystallized from EtOH as needles, m.p. 215–217°. This compound gave a positive Craven test[8] and violet color with 10% NaOH.[14] ν_{max} 1750, 1700, 1658 and 1635 cm^{-1}. NMR peaks at δ 3·85 and 3·90 p.p.m. (OCH_3 groups). (Found: C, 65·3; H, 3·9; O, 30·6. $C_{17}H_{12}O_6$ required: C, 65·4; H, 3·9; O, 30·7.)

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