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STUDIES ON PAKISTANI ARTEMISIAS.
Part I. Isolation of a Coumarin from *Artemisia elegantissima*, and its Structure Determination

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A coumarin has been isolated from *Artemisia elegantissima* from Islamabad area in Pakistan. With UV, IR, NMR and Mass spectroscopic studies, its structure is shown to be 6,7-dimethoxycoumarin.

INTRODUCTION

Artemisia, a very large genus of the *compositae* family has about 280 species – small shrubs – growing in the northern hemisphere in the arrid regions [1], over thirty of which occur in Pakistan. Of these, *Artemisia maritima* assumed a special significance as this plant, growing in the Kurram Valley, was the richest source in the world (a variety growing in Russia is reported to be even richer) of a very potent and hence commercially important anthelmintic "santonin" (a sesquiterpene lactone). A factory – M/s Kurram Chemicals – established nearly 50 years ago in Rawalpindi, was extracting santonin, for export, and was earning a substantial amount of foreign exchange for the country. Lately export of santonin has considerably decreased on account of competition from cheaper and less toxic synthetic anthelmintics. This has necessitated the need for finding alternate uses of santonin, or development of useful products by modification of its structure, as well as to have look at other species of this plant for discovering more useful medicinal agents.

Artemisia elegantissima [2] (Syn. Artemisia indica) occurs in Islamabad, Chitral and Kashmir areas of Pakistan. As no work has been reported on this species, therefore, this herb was collected from Islamabad to study its chemical constituents. The plant extracts were free of santonin. Extraction of the aerial parts of the herb with chloroform and concentration of the extract yielded a colourless crystalline material, m.p. 138-140°, which appeared to be the main product from the plant. High resolution mass spectrum of the compound gave the molecular ion formula as $C_{11}H_{10}O_4$ which was also indicated by its elemental analysis.

The compound burnt with a sooty flame, and showed very strong absorption in the carbonyl region of its IR spectrum. It was neutral in character. However, it dissolved in dilute aqueous alkali, and was reprecipitated on acidification which indicated it to be an aromatic lactone, possibly a coumarin.

The carbonyl stretching frequency in coumarins [3a] (α pyrones) occurs in the region 1700–1750 cm^{-1} and absorptions at 1722, 1610 and 1552 cm^{-1} are also characteristic of coumarins [4]. The isolated compound also shows strong carbonyl absorption at 1710 cm^{-1} and absorptions at 1610 and 1560 cm^{-1} further indicated it to possess a coumarin nucleus.

The NMR spectrum of the compound shows two singlets (3 protons each) at δ 3.9 and δ 3.92 corresponding to the two methoxyl groups and two doublets at δ 6.27 and δ 7.62, each corresponding to one proton, with the same coupling constant of 9.5 Hz. In addition, two singlets occur, each at δ 6.7 and δ 6.75. Appearance of a pair of doublets with a coupling constant of 9.5 Hz, centred at δ 6.1 – 6.4 and δ 7.5 – 8.3 in the NMR spectrum (recorded in deuterochloroform) of a natural product strongly indicates a coumarin unsubstituted in the pyrone ring [3a, 5]: these characteristic signals arise from hydrogens attached to C-3 and C-4 respectively, and absorption in the range δ 7.5 – 8.3 occurs due to C-4 hydrogen [3b]. Doublets observed thus at δ 6.27 and δ 7.62 in the isolated compound appear to be due C-3 and C-4 hydrogens of a coumarin structure. Lack of the significant splitting of the two aromatic protons arising near δ 7.0 indicates presence of hydrogens at the para positions in the aromatic ring at C-5 and C-8, and is characteristic of a 6,7-di-substituted coumarin [4]. The two singlets thus at δ 6.70 and δ 6.75 in the isolated compound appear to be due to C-5 and C-8 hydrogens of a coumarin nucleus. Aromatic methoxyl groups normally resonate in the range δ 3.8 – 4.4 in the substituted coumarins therefore two singlets arising at δ 3.9 and δ 3.92 in the NMR spectrum of the isolated compound appear to be due to the aromatic methoxyl groups. The NMR spectrum thus indicated strongly 6,7-dimethoxycoumarin (1) to be the structure of the isolated compound.

The presence of 7-methoxycoumarin chromophore moiety is indicated by UV absorptions [4] near 227, 253, 297 and 327 nm. Scopoletin (6-methoxy-7 hydroxycoumarin) (2) and isoscopoletin (6-hydroxy-7-methoxy-

coumarin) (3), show absorption at 228, 252, 260, 295, 344 nm and 229, 253, 260, 295, 350 nm respectively [3c]. The UV spectra of these compounds are very similar to that of the isolated compound which shows absorptions at 220, 225, 250, 272, 295, 310 and 340 nm. It is known that there is a great similarity in the UV spectra between the hydroxyl derivatives and their ethers [6], and 6,7-dimethoxycoumarin shows a more complicated UV spectrum than that of coumarin [7]. The UV spectrum of the compound thus also strongly favours 6,7-di-methoxycoumarin structure.

The high resolution mass spectrum of the isolated compound shows characteristic peaks at *m/e* 206 (M^+; 100 %); *m/e* 191 (M^+-CH_3 : 46.76 %); *m/e* 178 (M^+-CO; 18.19 %); and *m/e* 163 ($M^+-[CO+CH_3]$; 30.45 %) corresponding to $C_{11}H_{10}O_4$, $C_{10}H_7O_4$, $C_{10}H_{10}O_3$ and $C_9H_7O_3$ ions respectively. It is known that compounds derived from coumarins loose oxygen as carbon monoxide under electron impact and usually form a stable benzofuran ion [8]. The high resolution mass spectrum of the ions shows that they possess the structures 4 to 7.

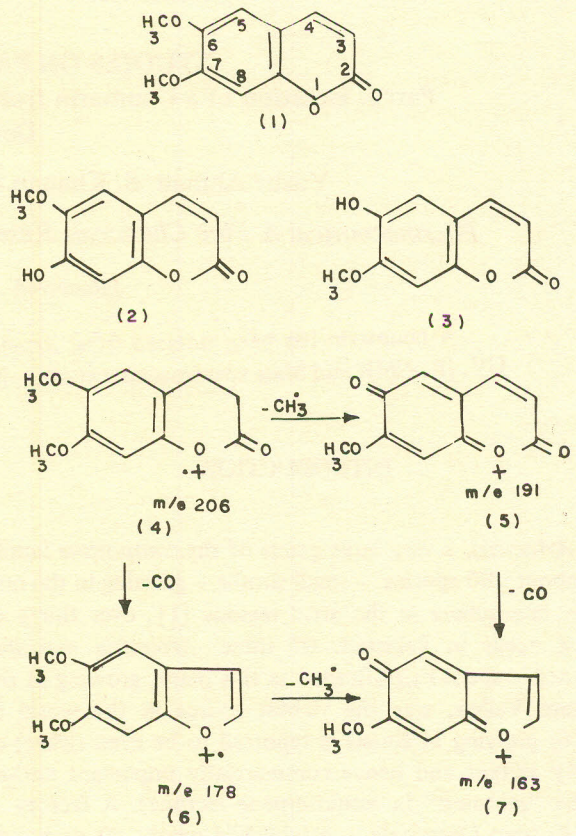
Thus through high resolution mass spectroscopy giving very accurate molecular weights of the fragmentation ions, we have confirmed the earlier observations of Djerassi [9] who also has reported the mass spectrum of 6,7-dimethoxycoumarin (1) and observed the molecular ion peak to be the most intense (100 %) and for the other ions appearing at *m/e* 191, *m/e* 178 and *m/e* 163, he suggested the structures 4 to 7.

The above data thus finally established that the compound, isolated from *A. elegantissima* of Islamabad area, is 6,7-dimethoxycoumarin (1).

6,7-Dimethoxycoumarin (scoparone [10], esculetin dimethyl ether [11], 6,7-dimethylesculetin [12]) also occurs in *A. scoparia* [10, 13-16] and *A. capillaris* which are components of the Chinese drugs [17], Ing. chen-hao and Inchinko-to respectively which are used for treatment of jaundice in Chinese medicine. 6,7-Dimethoxycoumarin (1), a component of these drugs, increased the bile secretion and appeared to be responsible for the therapeutic activity of the above crude Chinese drugs [17].

6,7-Dimethoxycoumarin (1) also occurs in *A. compestris, A. dracunculoides* Pursh, *A. dracunculus* L., *A. parviflora,* Roxb. and *A. tridentata,* var. *vaseyana* (Rydb.) Beetle [3d].

Recently lot of interest has been shown in the pharmacological activity of 6,7-dimethoxycoumarin (1). Besides possessing Choleretic activity [17, 18], it also shows hypotensive [18–21) activity but its anthelmintic action is week [22]. It also shows anti-inflammatory and analgesic properties in addition to choleretic activity [18]. More work is thus planned on *A. elegantissima* to develop the medicinal uses for the crude herb and 6,7-dimethoxycoumarin (1).



EXPERIMENTAL

The herb was collected from Islamabad 1–9 area and Chak Shahdad near the city of Islamabad on the morning of September 14, 1981. The collected plants were dried in a shed and the dried stems were removed before extraction of the remaining powdered aerial parts.

IR. spectra were recorded on a JASCO IR IA spectrophotometer. UV. spectra were recorded on a Shimadzu UV-240 spectro-photometer. The NMR spectra were obtained on Bruker WP 100 SY 100 mHz. FT– ^{13}C and ^1H NMR spectrometer. Mass spectra were recorded on a MAT 312 High Resolution Mass Spectrometer. Microanalyses were performed on a Karlo-Erba Elemental Analyser 1106. Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Thin layer chromatography was carried out on silica gel plates.

Extraction of Artemisia elegantissima *Plants.* Dried *Artemisia* herb (13 g) from Islamabad 1–9 area was extracted with chloroform (400 ml) in a soxhlet apparatus for 8 hours. Evaporation of chloroform extract *in vacuo* gave a dark green residue. It was dissolved in ethanol (10 ml) and then water (50 ml) was added followed by addition of charcoal (0.9 g). The solution was then refluxed for a few minutes, cooled to room temperature and filtered.

The filtrate was extracted with chloroform (3 x 33 ml) and chloroform extract was dried with anhydrous sodium sulphate. Evaporation of the dried chloroform extract gave a colourless solid (190 mg; 1.46 %), m.p. 130–140°. It was recrystallized from ethanol to obtain pure 6,7-dimethoxycoumarin (105 mg; 0.8 %), m.p. 137–139° (Lit. [23] m.p. 142–143°) giving only one spot on thin layer chromatography (chloroform-ethyl acetate, 1:1). It was soluble in hot water. IR (K Br): 1710(s), 1610(m), 1560(w), 1520(w), 1450(w), 1420(w), 1380(w), 1270(s), 1250(s), 1200(w), 1165(w), 1140(m), 1100(w), 1030(w), 990(w), 840(w), 810(w), 740(w) cm^{-1}; λ_{max} in MeOH (log ϵ): 220 (4.34), 225 (4.3), 250 (3.95), 272 (3.93), 295 (3.95), 310 (3.95), 340 (4.05) nm; NMR ($CDCL_3$) δ : 3.9 (S, 3H), 3.92 (S, 3H), 6.27 (d, 1H; J = 9.5 Hz), 6.7 (S, 1H), 6.75 (S, 1H), 7.62 (d, 1H, J = 9.5 Hz) MS *m/e* : 206.0587 (M^+, 100 %) : C_{11} H_{10} O_4), 191.0343 (M^+-CH_3, 46.76 %; C_{10} H_7 O_4), 178.0627 (M^+-CO, 18.19 %; C_{10} H_{10} O_3), 163.0392 (M^+-CO & CH_3, 30.45 %; C_9 H_7 O_3). Anal. Found : C, 61.46 %; H, 5.15 %; Calcd. for C_{11} H_{10} O_4, 0.5 H_2O; C, 61.39 %, H, 5.11 %.

Artemisia elegantissima sample from Chak Shahdad was also extracted by the same procedure. This sample (13 g) gave 250 mg, (1.92 %) of colourless crystals on recrystallization of the residue, obtained on evaporation of chloroform extract, and this material melted at 133–136°. Another recrystallization afforded more pure product (120 mg; 0.92 %), m.p. 138–140°, which shows the same infrared spectrum as that of 6,7-dimethoxycoumarin from Islamabad 1–9 area.

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