VACCAXANTHONE, A NOVEL XANTHONE ACID FROM SAPONARIA VACCARIA

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Abstract- Vaccaxanthone (1), a novel 7-carboxy-1,3,5,8-tetraoxygenated xanthone has been isolated from Saponaria vaccaria. Its structure has been established from spectroscopic data including uv, ir, high resolution-ms, ^{1}H- and ^{13}C-nmr as 1,8-dihydroxy-3,5-dimethoxyxanthone-7-carboxylic acid.

Saponaria vaccaria (Syn. Vaccaria pyramidata, V. segetalis, V. hispanica) is an annual herb growing as weed in cultivated fields in Northern Areas of Pakistan and also widespread in the flora of USSR.[1] It is reputed to possess sudorific, emetic, and laxative properties and is also used in the indigenous system of medicine for the treatment of jaundice, rheumatism, hepatic eruption, and venereal ulcers.[2] The toxicity against worms, paramecium, and other unicellular organisms is also reported.[3] Although enormous work has been done on various parts of this plant[4-7] but uptill now no xanthone has so far been reported from genera Saponaria and Vaccaria.

Preliminary tlc examination of the methanolic extract of the total plant material showed the presence of many phenolic compounds, one of which has now been isolated by column chromatography over silica gel and named vaccaxanthone. The chemical and spectral studies showed it to be a distinguishing variant of the 1,3,5,8-tetraoxygenated xanthone system.

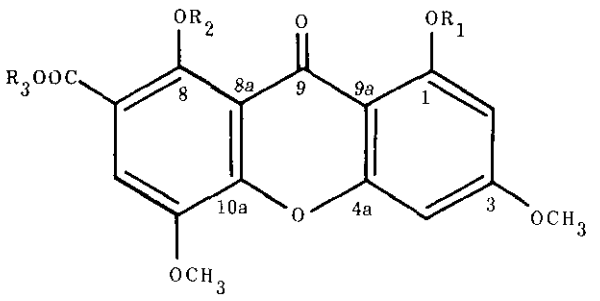
RESULTS AND DISCUSSION

Vaccaxanthone (1) formed yellowish viscous oil, $[\alpha]_D$ - 15.37° (c=0.2,$CHCl_3$), and gave blue coloration with ferric chloride. Methylation of 1 with dimethyl sulfate and sodium hydride in tetrahydrofuran formed methylate 2 (M^+ at m/z 374.0981, $C_{19}H_{18}O_8$ requires 374.1001). Acetylation of 1 with acetic anhydride and pyridine provided diacetyl derivative (3) (M^+ at m/z 416.0724, $C_{20}H_{16}O_{10}$ requires 416.0742). These reactions indicated the presence of two phenolic and one carboxylic groups.

The uv spectrum showed the presence of 1,3,5,8-tetraoxygenated xanthone system,[8] showing maxima at 230, 254, 278, 300, and 334 nm. The ir spectrum indicated the presence of carboxyl group (very broad band between 3400-2600 cm^{-1} and C=O stretching at 1700 cm^{-1}), conjugated carbonyl (1660 cm^{-1}) and conjugated C=C (1620 cm^{-1}).

High resolution mass spectrum (hrms) of 1 afforded the molecular ion at m/z 332.05250, corresponding to the molecular formula $C_{16}H_{12}O_8$, revealing eleven degree of unsaturation in the molecule. The molecular ion was confirmed by negative FAB mass spectrometry. The broad band decoupled ^{13}C-nmr spectrum revealed sixteen carbons, their multiplicities were determined through DEPT pulse sequence (keeping the last polarization pulse angle θ = 45°, 90°, 135°).[9,10] It showed the carbonyl carbon of the carboxyl group at 167.64 ppm, while the signal of the conjugated carbonyl at 184.10 ppm was characteristic for a doubly chelated carbonyl (1,8-di-OH).[8] Xanthones carrying hydroxyl groups at positions 3 and 5 are readily soluble in dilute Na_2CO_3 solution and show bathochromic shift of the K-band in uv spectrum on addition of NaOAc.[11] The absence of these effects in 1 further supported the presence of hydroxyl groups at positions 1 and 8. The ^{13}C-nmr spectrum further revealed signals of two methoxyl carbons (δ 57.42 and δ 55.85), three methine carbons (δ105.59, δ 97.28, and δ 93.01), nine quaternary carbons, three of which had no oxygen substituent (δ132.66, δ 102.53, and δ 99.97) and six of which had an oxygen substituent(δ 166.23-143.13). The presence of 1,3-dioxygenated polyketide with a hydroxyl group at C-1 on the ring B was concluded from the multiplicity of the C-2 and C-hydroxyl carbon signal in the ^{1}H-coupled ^{13}C-nmr spectrum of 1.[12] The long range multiplicity of the C-2 signal was a double doublet because of the coupling to the C-1 hydroxyl proton and the C-4 proton. The C-hydroxyl carbon appeared as a triplet due to coupling to C-1 hydroxyl proton and the C-2 proton. These long range multiplicities were same as C-2 and C-hydroxyl carbon signals in the ^{1}H-coupled spectra reported in literature[8] for 1,8-dihydroxy-3,5,6,7-tetramethoxy-xanthone and 1-hydroxy-3,5,6,7,8-pentamethoxy-xanthone, respectively.

One of the methoxyl group at position 3 was also reflected in the ^{1}H-nmr spectrum showing meta coupling of 2.29 Hz between H-2 and H-4. The ^{1}H-nmr spectrum further showed 2H singlet at δ 11.57 a 1H singlet at δ 11.48 and two sharp methoxyl singlets at δ 3.88 and δ 3.93. The 1H singlet at δ 11.48 was assigned to one of the phenolic groups while the 2H singlet at δ 11.57 was due to the other phenolic group and the carboxylic proton. The superimposibility of these two signals was clearly revealed by the ^{1}H-nmr spectrum of the diacetyl derivative 3 in which 1H sharp singlet of carboxyl proton was observed at the same position. The position 5 had already been indicated for the methoxyl group by the uv spectrum; consequently the carboxyl group must be at position 6 or 7. The downfield shift of one of the chelated hydroxylic groups strongly indicated the presence of the carboxylic group at the adjacent position 7. This was further authenticated by chemical shift of the proton at C-6 (δ 7.27) which showed complete agreement with that observed earlier by Sluis and Labadie.[8] The structure of vaccaxanthone (1) and its derivatives, 2 and 3, could therefore be represented by Figure 1.



1 $R_1 = R_2 = R_3 = H$

2 $R_1 = R_2 = R_3 = CH_3$

3 $R_1 = R_2 = COCH_3$, $R_3 = H$

FIG. 1

The isolation of vaccaxanthone constituted the first example of the natural occurrence of a free carboxyl derivative of 1,3,5,8-tetraoxygenated xanthone and its isolation may be of chemotaxonomic significance.

EXPERIMENTAL

Uv and ir spectra were recorded on Shimadzu UV-240 and JASCO A-302 spectrophotometers, respectively. Optical rotation was recorded on JASCO Model DIP-360, Digital Polarimeter, Ogawaseiki Co. Ltd. Hrms were recorded on Finnigan MAT-312 mass spectrometer connected to PDP 11/34 (DEC) Computer System. Nmr spectra were recorded on a Bruker AM-300 spectrometer with TMS as internal reference. Tlc experiments were performed on silica gel (PF-254, 0.2 mm) plates (E.Merck).

Isolation of vaccaxanthone (1): The plant material (stem, 20 kg) was collected from the Northern areas of Pakistan and was identified by a Plant Taxonomist, Department of Botany, University of Karachi. The plant material was chopped into small pieces and percolated with MeOH (60 l) for 3 days at room temperature. After obtaining three extracts, the combined methanolic extract was evaporated under reduced pressure to yield darkish brown gummy residue (300 g). A part of it (35 g) was chromatographed over silica gel (1.4 kg) eluting with various solvent gradients of increasing polarities. Vaccaxanthone (1) (45 mg) was eluted with n-hexane-chloroform (50:50) eluant as viscous oil and was further purified by preparative tlc using solvent system n-hexane-chloroform (20:80). Besides vaccaxanthone, a number of other natural products were obtained

from the column out of which stigmasterol (40 mg) and three glycosides namely vacsegoside B (10 mg), vacsegoside C (8 mg), and vaccarine (15 mg) could be obtained pure and characterised. These have already been reported from S.vaccaria.[4-6,13]

Uv λ_{max}^{EtOH} nm: 230, 254, 278, 300, and 334; ir $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400-2600 and 1700 (COOH), 1660 (conjugated carbonyl), 1620 (conjugated C=C), 1250 (OCH_3); hrms m/z: M^+, 332.0525 ($C_{16}H_{12}O_8$); **^{1}H nmr** ($CDCl_3$) δ : 11.57 (2H, s, 8-OH and COOH), 11.48 (1H, s, 1-OH), 7.27 (1H, s, H-6), 6.53 (1H, d, J_{meta} = 2.26 Hz, H-4), 6.37 (1H, d, J_{meta} = 2.26 Hz, H-2), 3.93 (3H, s, OMe), 3.88 (3H, s, OMe), **^{13}C nmr** ($CDCl_3$) δ : 184.10 (C-9), 167.64 (COOH), 166.23 (C-3), 162.49 (C-1), 158.79 (C-4a), 154.43 (C-8), 150.87 (C-5), 143.13 (C-10a), 132.66 (C-7), 105.59 (C-6), 102.53 (C-8a), 99.97 (C-9a), 97.28 (C-2), 93.01 (C-4), 57.42 [OMe(5)], 55.85 [OMe(3)]. The *assignments were made through comparison with published spectra of xanthones.*[8,14,15]

Methylation of vaccaxanthone (1): Vaccaxanthone (5 mg) was stirred at room temperature with dimethyl sulfate (0.2 ml) and excess sodium hydride in tetrahydrofuran (5 ml). After 4 h dimethyl sulfate (0.25 ml) and one drop of water were added and the mixture was allowed to stand overnight. The solution was added to 10% sodium hydroxide (25 ml) and heated on the steam bath for 1 h. The basic solution was cooled and extracted with dichloromethane. The organic layer was washed with water, dried over anhydrous sodium sulfate, and evaporated to get methylated product which was purified by preparative tlc using solvent system n-hexane-chloroform (15:85) to afford 2 as light yellow oil (1.70 mg); **hrms** m/z: M^+, 374.0981 ($C_{19}H_{18}O_8$); **^{1}H-nmr** ($CDCl_3$) δ : 7.24 (1H, s, H-6), 6.53 and 6.36 (each 1H, d, J_{meta} = 2.27 Hz, H-4 & H-2), 4.11 (3H, s, COOMe), 4.08, 3.99, 3.94, 3.88 (each 3H, s, OMe).

Acetylation of vaccaxanthone (1): Vaccaxanthone (1) (5 mg) was dissolved in pyridine (1.0 ml) and refluxed with acetic anhydride (2.5 ml) for 30 min. The reaction mixture was worked up in the usual manner to afford an oily residue which showed one major spot on tlc along with traces of impurities. It was purified by preparative tlc using solvent system n-hexane-chloroform (15:85) to yield compound 3 as light yellow oil (4.0 mg); hrms m/z: M^+, 416.0724 ($C_{20}H_{16}O_{10}$); ^{1}H nmr ($CDCl_3$) δ : 11.56 (1H, s, COOH), 7.21 (1H, s, H-6), 6.50 and 6.33 (each 1H, d, J_{meta} = 2.27 Hz, H-4 & H-2), 3.90 and 3.86 (each 3H, s, OMe), 2.47 and 2.43 (each 3H, s, Ac).

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