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RESEARCH ARTICLE

Coumarins From The Roots of *Clausena pentaphylla*

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Summary

Three coumarins were isolated from the roots and stem bark of Clausena pentaphylla. Analysis of their spectral data (UV, IR, MS, ^{1}H, ^{13}C – NMR experiments) confirmed their structures as 3, 10-bis (1, 1-dimethyl prop-2-en-1-yl) -5, 6, 7-trihydroxy-8, 8-dimethyl-7, 8-dihydro pyranochromen-2-one, bergapten and xanthotoxin.

***Key Words**: Rutaceae, Clausena pentaphylla, Coumarins*

Clausena pentaphylla köklerinden elde edilen kumarinler

Özet

Clausena pentaphylla kök ve dal kabuklarından üç kumarin izole edilmiştir. Spektral değerlerin (UV, IR, MS, 1H, 13C-NMR) analizi sonucu yapılar 3,10 bis (1,1-dimetil prop-2-en-il)-5,6,7-trihidroksi-8, 8-dimetil-7, 8-dihidro piranokromen-2-on, bergapten ve ksantotoksin olarak belirlenmiştir.

***Anahtar Kelimeler:** Rutaceae, Clausena pentaphylla, Kumarinler*

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INTRODUCTION

The genus *Clausena* belongs to the family Rutaceae of the order Rutales and is in the subtribe Clauseninae. The subtribe Clauseninae contains three genera, *Glycosmis*, *Clausena* and *Murraya*, having very simple, more or less primitive flower and fruit structures. *Clausena pentaphylla* is one of the important medicinal plants found broadly in the district Shahjahanpur, commonly known as *ratanjote*, *rowana*, *surjmukha*, *teyrar* in India (1). The plant was identified by the Department of Botany of

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G. F. College (Rohilkhand University) Shahjahanpur. It has been widely used as a folk medicine for the treatment of diaohorrea, renal colic, vesical pain, renal calculas and pain of hepatic. It is also used in uterine pain and colic and in other disorders. Bark acts as anti-inflammatory, spasmolytic agent and used in veterinary medicine for wounds and sprains (1). A compound, clausmarin, isolated from *C. pentaphylla* showed sposmolytic activity and reduces blood pressure (1-3). Previous phytochemical studies have indicated that *Clausena* species contain structurally diverse and biologically active carbazole alkaloids and coumarins (1, 4-7).

MATERIALS AND METHODS

General procedures

Ultra violet absorption spectrum was recorded on Perkin-Elmer Lambda Bio 20 UV spectrometer. IR spectroscopy was performed using the KBr disc method on Perkin-Elmer 1710 infrared Fourier transformation spectrometer. NMR spectra were recorded on Bruker AVANCE DRX- 300(300, 100 Hz). Chemical shifts are shown in δ values (ppm.) with tetramethylsilane (TMS) as an internal reference. FABMS was recorded on JEOLSX 1021/DA-6000 mass spectrometer. Column chromatography was carried out using silica gel (60-120 mesh). Chemicals of analytical-reagent grade were purchased from E-Merck (India).

Plant material

The roots of *Clausena pentaphylla* were collected from the rural areas of the Shahjahanpur District in the month of September. Authentication was achieved by the comparison with the herbarium specimen deposited in the herbarium of the faculty of botany, G. F. College (Rohilkhand University), Shahjahanpur (Herbarium no: GFCB 1361). Fresh or dried plant material can be used as a source for the extraction of secondary plant components. Freshly harvested and dried material is directly used since old dried material stored for a period may undergo some qualitative changes.

Extraction and Isolation

Dried and pulverized bark and leaves (1.5 kg) of *Clausena pentaphylla* were first extracted with petroleum ether (3L x 5 times) and then was evaporated under vacuum on rotary evaporator below 50°C temperature to yield a brownish mass (53 g). The mass was then subjected to column chromatography. A well-stirred suspension of silica gel (100 -150 g in petroleum ether 60-80°) was poured into the column (150 cm long and 50 mm in diameter). When the absorbent was well settled, the excess of petroleum ether was allowed to pass through the column. The column was eluted with the petroleum ether, chloroform, EtOAc and methanol and their mixtures of increasing polarity. Elution, with petroleum ether: $CHCl_3$ (1: 3) afforded CP-1 (0.84 mg), with chloroform: EtOAc (2:7) furnished CP-2 (1.02 mg) and with chloroform: EtOAc (5:9) provided CP-3, 10-bis (1, 1-dimethyl prop-2-en-1-yl) -5, 6, 7-trihydroxy-8, 8-dimethyl-7, 8-dihydro pyranochromen-2-one (0.51 mg), respectively.

RESULTS AND DISCUSSION

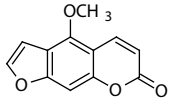
The compound CP-3 was isolated as colorless semi-solid compound from the petroleum ether extract. The UV spectrum exhibited the absorption maxima at 215, 265, 287 and 332 nm, suggesting that the compound belong to the coumarin family. Its IR spectrum showed absorption bands at 3356, 1699, 1620 and 1570 cm^{-1} also in support that the compound was a coumarin derivative (8).

The ^1H NMR studies showed it to be coumarin, with two 1, 1-dimethylallyl groups, to which a pyrone ring attached at C-6 and C-7 (8). The spectrum also demonstrated well-resolved signals of the side chain protons. Two singlets observed at δ 1.46 and δ 1.61 were attributed to two -C $(CH_3)_2$ systems, permitted the assignment of C_5H_9 fragment as two 1, 1-dimethylallyl groups. One-proton singlet at δ 7.89 was attributed to pyran ring proton (H-4). The singlet nature of H-4 suggested the attachment of the one C_5H_9 chain at C-3 of the coumarin (8-10)

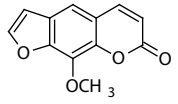
The locations of two 1, 1-dimethylallyl groups were determined by HMBC spectrum simultaneously, which showed cross peaks [H-4 (δ 7.89) to C-5 (δ 151.9)/ C-8a(δ 153.1)/C-2 (δ 162.8)/C-1'(δ 39.7),

C-1′ (δ 39.7) to H-1′ -Me(δ 14.5) /C-2′ (δ 145.3) /C-3′ (δ 5.03) /C-3′ (δ 5.04) and C-8(δ 113.2) to H-1′′Me(δ 1.61) / H-1′′ Me(δ 1.57) / H-2′′ (δ 6.19) /H-3′′ (δ 4.85) /H-3′′ (δ 4.79)]. These data suggested that one 1, 1-dimethylallyl group positioned at C-3 while the other at C-8, respectively (8-10).

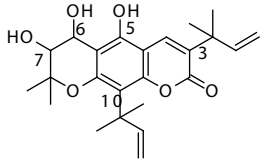
In NMR spectrum signals observed at δH 4.83, 3.71 and δH 1.21, 1.46 coupled with the signals at δC 78.9, 75.1, 69.8, 17.8 and δC 29.1 suggested the presence of 3,4-dihydroxy-2,2-dimethyl at C-6 and C-7 of the coumarin nucleus.



CP-1



CP-2



CP-3

On the basis of these spectral data the compound CP-3 was identified as 3, 10-bis (1, 1-dimethyl prop-2-en-1-yl) -5, 6, 7-trihydroxy-8, 8-dimethyl-7, 8-dihydro pyranochromen-2-one (9). CP-3 was isolated from the genus Clausena for the first time in this study. Compounds CP-1 and CP-2 were two known compounds, whose UV, IR, MS, ^{1}H NMR and ^{13}C NMR data are in close agreement with bergapten and xanthotoxin in the literatures (10, 11).

Compound CP-1: (bergapten) white crystals, m p =188-190 °C; UV (MeOH) λ_{max}/nm: 210, 260 and 310; ^{1}H NMR ($CDCl_3$) δ/ppm: 8.21 (1H, d, *J*=9.8 Hz, H-4), 7.71 (1H, d, *J*=2.8 Hz, H-7), 7.16 (1H, s, H-9), 7.10 (1H, d, *J*=2.8 Hz, H-6),6.30 (1H, d, *J*=9.8 Hz, H-3) and 3.82 (3H, s, OCH_3);^{13}C-NMR ($CDCl_3$) δ/ppm: 21.9 (9-CH_3), 60.1 (4-OCH_3), 105.0 (C-3), 106.4 (C-4a),112.5 (C-6), 112.7 (C-3a), 139.2 (C-5), 144.8 (C-2), 149.6 (C-4), 152.7 (C-8a), 158.4 (C-9a), 161.1 (C-7)

Compound CP-2: (xanthotoxin) white crystals, mp 146-147 °C; ^{1}H-NMR ($CDCl_3$,300 MHz) δ/ppm: 4.29 (s, 3H, 9-OCH_3), 6.35 (d, *J*=9.5 Hz, 1H, H-6), 6.82 (d, *J*=2.0 Hz, 1H, H-3), 7.34 (s, 1H, H-4), 7.69 (d, *J*=2.0 Hz, 1H, H-2), 8.10 (d, *J*=10.0 Hz, 1H, H-5). ^{13}C NMR ($CDCl_3$,100 MHz)) δ/ppm: 61.2 (9-OCH_3),107.7 (C-3), 112.9 (C-4), 114.7 (C-6), 116.5 (C-4a), 126.1 (C-3a), 132.8 (C-9),143.0 (C-8a), 144.3 (C-5), 146.6 (C-2), 147.7 (C-9a), 160.4 (C-7)

Compound CP-3: colorless semi-solid; $C_{24}H_{30}O_6$; UV(MeOH) λ_{max}/nm: 215, 265, 287 and 332; IR(KBr) ν_{max}/cm^{-1}: 3356, 1699, 1620 and 1570; MS *m/z* (%): 414$[M]^+$ (39), 396$[M - H_2O]^+$ (100), 381 $[M - H_2O - Me]^+$ (75), 327 (14), 299 (42); ^{1}H NMR ($CDCl_3$) δ/ppm: 7.89 (1H,s, H-4), 6.13 (1H, d, *J* = 17.3, 10.2 Hz, H-2′), 5.04 (1H, d, *J* = 17.3 Hz, H-3′), 3.68 (1H, d, *J* = 8.1 Hz, H-10), 4.91 (1H, d, *J* = 8.1 Hz, H-11), 5.03 (1H, d, *J* = 10.2 Hz, H-3′), 6.19 (1H, d, *J* = 17.4, 10.3 Hz, H-2′′), 4.79 (1H, d, *J* = 17.4 Hz, H-3′′), 4.85 (1H, d, *J* = 10.2 Hz, H-3′′), 1.51 (3H,s, 9-Me), 1.23 (3H,s, 9-Me), 1.45 (6H,s, 1′-Me), 1.61 (3H,s, 1′′-Me), 1.59 (3H,s, 1′′-Me); ^{13}C NMR ($CDCl_3$) δ/ppm: 162.8 (C-2), 127.9 (C-3), 134.3 (C-4), 104.3 (C-4a), 151.9 (C-5), 106.1 (C-6), 157.6 (C-7), 113.2 (C-8), 153.1 (C-8a), 78.9 (C-9), 18.9 (9- Me), 26.7 (9- Me), 76.4 (C-10), 70.4 (C-11), 39.7 (C-1′), 26.2 (1′-Me), 145.3 (C-2′), 111.8 (C-3′), 41.6 (C-1′′), 30.1 (1′′-Me), 31.1 (1′′- Me), 149.8 (C-2′′), 106.6 (C-3′′)

CONCLUSION

This is the first report describing the isolation of 3, 10-bis (1, 1-dimethyl prop-2-en-1-yl) -5, 6, 7-trihydroxy-8, 8-dimethyl-7, 8-dihydro pyranochromen-2-one from *Clausena pentaphylla*.

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