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PHYTOCHEMICAL STUDIES ON *CUSCUTA REFLEXA*

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Nine compounds including coumarin α-amyrin, β-amyrin, α-amyrin acetate, β-amyrin acetate, oleanolic acetate, oleanolic acid, stigmasterol and lupeol are reported for the first time from the genus *Cuscuta*. Their structures have been established with the help of spectral data.

Key words: *Cuscuta reflexa,* Coumarin, Triterpenes, Steroids.

Introduction

The genus *Cuscuta,* belonging to the family convolvulaceae, comprises of three species. All of the three are leafless, twining parasites with slender yellowish stems, distributed in tropical and temperate region. These are found in Karachi and other parts of Pakistan. *C. reflexa* is depurative and purgative (Shastri 1950). Internally, it is used in protracted fever and induration of liver while externally against itch and other skin diseases. It is applied locally as poultice. The literature survey revealed that no phytochemical work has been carried out on any species of genus *Cuscuta.*

The methanolic extract of the stems of *C. reflexa* showed positive cytotoxic activity in Brine-shrimp test. Further pharmacological screening revealed a very strong antimicrobial activity in the methanolic extract. This prompted us to carry out the bioassay directed isolation studies on this plant. Herein, we describe the isolation of a coumarin identified as 6,7, 8-trimethoxy -2H-1-benzopyran-2-one (1), stigmasterol (2), lupeol (3) α-amyrin (4), β-amyrin (5), oleanolic acetate (6), α-amyrin acetate (7) β-amyrin acetate (8) and oleanolic acid (9). None of these have previously been reported from the genus *Cuscuta.*

Experimental

The IR spectra was recorded on JASCO A-302 spectrometer. The mass spectrum were recorded on a Finnigan MAT112 spectrometer. The NMR spectra ($CDCl_3$): 300 MHz for ^1H and 125 MHz for ^{13}C unclei using TMS as int. standard. TLC: silica gel PF_{254}; CC: silica gel, 70-230 mesh.

Plant material: The plant material was collected from Karachi region and identified as *Cuscuta reflexa* Roxb. by Prof. M. Qaiser, Department of Botany, University of Karachi. A voucher specimen is deposited in Herbarium of the Department of Botany, University of Karachi.

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Isolation: The shade dried plant material was extracted thrice at room temperature with MeOH. The methanolic extract was partitioned between ethyl acetate and water. The ethyl acetate fraction was evaporated and divided into hexane soluble and insoluble fractions. The hexane soluble fraction was evaporated and the residue was chromatographed over silica gel using various mixtures of hexane, ethyl acetate and chloroform. It resulted in the isolation of eight pure compounds, α-amyrin, β-amyrin, α-amyrin acetate, β-amyrin acetate, oleanolic acid, stigmasterol, oleanolic acetate and lupeol.

The ethyl acetate insoluble fraction was further partitioned between butanol and water. The butanol fraction was evaporated and subjected to medium pressure liquid chromatography (MPLC) over silica gel 70-230 mesh using $CHCl_3$-methanol in increasing order of polarity. The fraction which eluted in $CHCl_3$: methanol (95:5), contained three spots. It was further separated by preparative TLC over silica gel using $CHCl_3$: methanol (9:1) as solvent system to obtain the UV active compound (1) 30 mg, m.p. 103-105°C, $[\alpha]_{D+}$+2.1° (C=0.41, $CHCl_3$): IR ($CHCl_3$) ν_{max} cm^{-1}: 2900, 1735, 1610, 1440, 1260, 1120, 1020, 810; ^1H NMR ($CDCl_3$): (500 mHz): δ7.93 (1H,d, J = 9.48 Hz H-4), 6.61 (1H, s, H-5), 6.20 (1H, δ, J = 9.48,H-3), 3.9 (3H, s, OMe), 3.8 (3H, s, OMe), 4.0 (3H, s, OMe); ^{13}C NMR ($CDCl_3$); δ (125 MHz), δ 56.20 (OMe), 61.23 (OMe), 61.82 (OMe) 95.45 (C-5), 153.36 (C-6), 138.60 (C-7), 152.0 (C-8) 106.99 (C-10), 150.1 (C-9); EIMS *m/z* (rel. int %) 236.2 [$C_{12}H_{12}O_5$, M^+] (100), 221 $(C_{11}H_9O_5)^+$ (85), 178 $(C_9H_6O_4)^+$ (10), 161 $(C_9H_5O_3)^+$ (8), 137, $(C_7H_5O_3)^+$ (4), 95 $(C_5H_3O_2)^+$.

Stigmasterol (2): Isolated from fraction which was eluted in hexane-chloroform (1:1) from CC crystallized from methanol, m.p. 168-169°C; $[\alpha]_D$= -50° ($CHCl_3$); EIMS *m/z:* 412.4 [$C_{29}H_{40}O$, M^+]. The physical and spectral data coincided with literature (Rubinstein *et al.*1976).

Lupeol (3): The fraction eluted in hexane-$CHCl_3$ (8:2) yielded a mixture. By repeated CC using the solvent system hexane-chloroform (6:4), pure (3) was obtained, m. p. 214-215°C, $[\alpha]_D = +27°$ ($CHCl_3$); EIMS *m/z* 426.4 [$C_{30}H_{50}O$, M^+]. The physical and spectral data coincided with literature (Brindopke and Ramesh 1978).

α-Amyrin (4): The fraction eluting in n-hexane-$CHCl_3$ (7:3) was further chromatographed over silica gel, and elution with hexane-$CHCl_3$ (6.5:3.5) gave uniform compound (4) from head fractions which crystallized from methanol, m.p. 184-185°C; $[\alpha]_D$+ 96° ($CHCl_3$); EIMS *m/z* :426.3 [$C_{30}H_{50}O$, M^+]. The physical and spectral data identified it as α-amyrin (Monaco and Previtera 1964; Seo *et al.* 1975).

B-amyrin (5): The fraction eluting in n-hexane-$CHCl_3$ (7:3) from CC was rechromatographed over silica gel and eluted with hexane-$CHCl_3$ (6.5:3.5) to give (5) from the tail fractions. It crystallized from methanol, m.p. 197-189°C; $[\alpha]_D$ + 99° ($CHCl_3$); EIMS *m/z:* 426.3 [$C_{30}H_{50}O$, M^+]. The physical and spectral data identified it as β-amyrin (Shamma *et al* 1962; Bryce *et al* 1967).

Oleanolic acetate (6): The fraction eluting in hexane-$CHCl_3$ (7:3) was further chromatographed over silica gel. The eluates obtained from hexane-$CHCl_3$ (6:4) afforded pure (6), m.p. 256-258°C, $[\alpha]_D = +74°$ ($CHCl_3$); EIMS m/z: 498.7 [$C_{30}H_{50}O_4M^+$]. The physical and spectral data corresponded to those reported in literature for oleanolic acetate (Shamma *et al* 1962).

α-amyrin acetate (7): The fraction eluting in n-hexane-$CHCl_3$ (2:8) from CC was again subjected to column chromatograph using hexane - $CHCl_3$ (1:9) as eluent to obtain (7) from head fractions as colourless crystals; m. p. 225 226°C; $[\alpha]_D$ +77° ($CHCl_3$); EIMS *m/z:* 468.5 [$C_{33}H_{52}O_2$, M^+]. The physical and spectral data coincided with literature (Heupel 1985; Abranovitch and Micetich 1963).

β-amyrin acetate (8): The fraction which eluted in n-hexane -$CHCl_3$ (2:8) from CC was rechromatographed over silica gel and elution with hexane-$CHCl_3$ (1:9) provided (8), from tail fractions. It crystallized from methanol, m. p. 244-245°C; $[\alpha]_D$ +69.9° ($CHCl_3$); EIMS *m/z:* 468.6 [$C_{30}H_{52}O_2$, M^+]. The physical and spectral data coincided with those described in literature for β-amyrin acetate (Awasthi and Mitra 1968; Chow and Quom 1970).

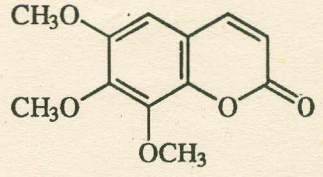
Oleanolic acid (9): The fraction which eluted in n-hexane-$CHCl_3$ (2:8) from CC was rechromatographed over silica gel. Elution with $CHCl_3$-methanol (9.8:0.2) provided pure (9)., m. p. 306-307°C, $[\alpha]_D$ +78.9° ($CHCl_3$); EIMS *m/z:* 456.3 [$C_{30}H_{48}O_2$, M^+]. The physical and spectral data showed complete agreement with the literature (Ageta and Ageta 1984).

Results and Discussion

Column Chromatography of the hexane soluble fraction of *Cuscuta reflexa* resulted in the isolation and characterization of six known triterpenes of oleane and urs series, namely, a-amyrin, b-amyrin, their acetates, oleanolic acid and their respective acetates. In addition, lupeol and stigmasterol were also obtained and characterized.

The less common coumarin (1) was isolated from the butanol soluble fraction through MPLC over silica gel. It was assigned the molecular formula $C_{12}H_{12}O_5$ by HRMS which showed $[M]^+$ peak at *m/z* 236.0627 (calc. 236.2240). The molecular ion peak was confirmed by positive FAB mass spectrum, which showed $[M+H]^+$ and $[M+Na]^+$ peaks at *m/z* 237 and 259, respectively.

The coumarin skeleton was evident by characteristic band in I R spectrum at 1735 cm^{-1} (C=O of α-pyrone) as well as presence of a typical AB system (δ 6.20 and 7.93, J = 9.48) for the H-3 and H-4 in ^1H NMR spectrum. Another aromatic singlet at δ 6.61 indicated trisubstitution oxygenation pattern on the aromatic nucleus. The presence of the methoxyl groups was reflected by sharp singles (δ 4.0, 3.9, 3.8). Association of the aromatic one proton singlet with only H-4 single in ^1H-NOE difference spectroscopy provided the strong evidence for the location of the aromatic proton at C-5. The ^{13}C NMR spectrum was in complete agreement to the assigned structure (Roitman and James 1985) and conclusive evidence was provided by HMQC and HMBC showing the long range coupling between H-5 C-5, C-6 and C-7. Thus compound (1) was indentified as 6, 7, 8-trimethoxy-2H-1-benzopyran-2-one. Previouslyly this compound has been isolated from *Fagara microphylla* (King *et al* 1954).



6, 7, 8-trimethoxy-2H-1-benzopyran-2-one.

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