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PYRROLIZIDINE ALKALOIDS FROM *ALKANNA ORIENTALIS* (L.) BOISS

By

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القلويدات البيروليزيدينية من نبات الكانا أورينتاليس

فايزة محمود حمودة و شمس الدين أمبابي اسماعيل
و ناهـد محمد حسن و وفاء توفيق و علاء كامل

تم فصل والتعرف على ثلاثة قلويدات هي : ٧ ـ أنجليل رترونسين ، ٩ ـ أنجليل رترونسين ، داي هيدروكسي تراي أنجيلورين من نبات الكانل أورينتاليس الذي ينمو في مصر . وذلك بالاضافة إلى بعض القلويدات الأخرى التي توجد بكميات صغيرة . القلويد ـ ٩ ـ أنجليل رترونسين فصل لأول مرة من جنس الكانا .

Key Words: Alkanna orientalis, Boraginaceae, Pyrrolizidine alkaloids, 7-Angelyl retronecine, 9-Angelyl retronecine, Dihydroxy-triangularine.

ABSTRACT

Three pyrrolizidine alkaloids; 7-angelyl retronecine (I), 9-angelyl retronescine (II), and dihydroxytriangularine (III) were isolated by DCCC and VLC from *Alkanna orientalis* growing in Egypt. Their structures were elucidated by MS, ^{1}H NMR and ^{13}C NMR spectral analyses. (II) (9-angelyl retronecine), was isolated for the first time from the genus *Alkanna.* In addition, three other minor alkaloids have also been detected by TLC.

INTRODUCTION

Alkanna orientalis belongs to the family Boraginaceae which is known to contain pyrrolizidine alkaloids (Bull *et al,* 1968). *A. tinctoria* was the only species reported to contain pyrrolizidine alkaloids (Roeder *et al,* 1984).

Alkanna species are characterized by their high content of quinonoid pigments in their roots (Afzal and Tofeeq, 1975). The plant is known to be used in folk medicine for the treatment of *ulcus cruris* and for wound healing (Papageorgiou, 1978). Several pharmaceutical preparations containing quinones from *Alkanna* have been reported (Papageorgiou, 1977). *Alkanna* species are represented in Egypt by two species viz. *A. tinctoria* which is very common and *A. orientalis* which grows in Sinai, Egypt (Taekholm, 1974). On reviewing the literature, *Alkanna* has been only investigated for its flavonoids (Mansour and Saleh, 1986). The present work deals with the investigation of the pyrrolizidine alkaloidal constituents of the plant.

EXPERIMENTAL

Materials:

DCCC was run on a Buchi 670 DCC chromatograph, at flow rate 40ml/h. VLC was carried out on a sintered glass funnel filled with 25 gm. alumina, Merck TLC grade. TLC was done on silica gel 60 PF plates (20 x 20), Merck and aluminium oxide 60 GF_{254} neutral type E, Merck. Mass spectra were recorded in Ei mode at 70 ev. ^{1}H NMR and ^{13}C NMR were run on Bruker WM 250 apparatus in $CDCl_3$ at 250.13 and 62.81 MHz respectively. The plant material was collected from St. Catherine, Sinai, Egypt.

Extraction and isolation of Alkaloids:

The dried powdered plant (7 kg) was extracted with about 70 l. 96% ethanol and evaporated *in vacuo*. The alcoholic extract was taken with an acidic solution of 10% HCl and kept overnight in the refrigerator and then filtered. The filtrate was extracted with diethyl ether (5 x 1 l.) and the aqueous acidic solution was shaken with 70 gm. Zn dust, kept overnight, filtered and then rendered alkaline with ammonium hydroxide (pH 9-10). The solution was then extracted with chloroform (6 x 2 l.) and the organic layers, after drying over anhydrous sodium sulphate, gave the total alkaloids (3.8 gm.). TLC of the total alkaloids on alumina run with C_6H_6: MeOH (9:1) + 5 drops ammonia solution showed the presence of six alkaloidal constituents after spraying with Dragendorff's reagent. The pyrrolizidine alkaloids possessed R_f values: 0.54 I, 0.68 II, 0.03 III, 0.93 IV, 0.87 V and 0.46 VI.

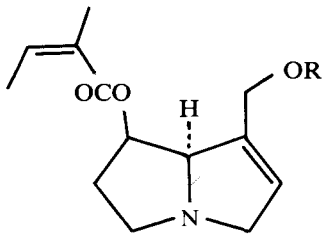
About 1.9 gm. of the total alkaloidal mixture was chromatographed by DCCC in the ascending mode using the solvent system C_6H_5Me: $CHCl_3$: MeOH: H_2O (5:5:7:2) (Otsuka *et al,* 1974). The chromatographic operation afforded a fraction containing a mixture of I and II. This fraction was further purified by preparative TLC on alumina through which the alkaloidal components were isolated and found to be chromatographically pure.

Another part (1.9 gm.) of the total alkaloidal mixture was separated by VLC following the method of Pelletier *et al* (1986) and Coll and Bowden (1986) in which aluminium oxide was used as an adsorbent and affecting with different solvents. Elution with toluene: chloroform (1:1) gave a fraction containing a mixture of I and II, while elution with $CHCl_3$ (100%) afforded a fraction containing a mixture of II and III. Further purification of the two fractions by preparative TLC yielded pure I (20 mg.), II (10 mg.) and III (24 mg.). Other alkaloidal fractions (IV, V and VI) were obtained in scanty amounts.

Spectral properties of the isolated alkaloids:

The following spectral data were obtained for the three identified alkaloids:

7-angelyl retronecine: (name used in the literature, another name also used is O^7-angelylretronecine)



7-Angelylretronecine
R = H

Mass spectrum: m/z (relative intensity)

237 (1.7), 219 (2.8), 191 (1.1), 154 (3.9), 149 (7.3), 137 (19.7), 111 (34.4), 106 (35.0), 94 (23.2), 124 (19.7), 80 (100).

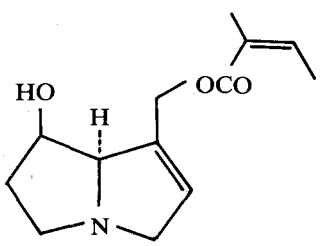
^1H NMR: ppm, J (Hz)

5.65, m, 1H, H-2, 3.47, m, 1H, H-3A, 4.05, m, 1H, H-3B, 2.76, m, 1H, H-5A, 3.40, m, 1H, H-5B, 2.19, m, 2H, H-6, 5.48, dd, 1H, J=3.5, J=2.0, H-7, 4.56, m, 1H, H-8, 4.19, s, 2H, H-9, 6.10, dq, 1H, J=7.5, J=1.5, H-12, 1.98, dq, 3H, J=7.0, J=1.5, H-13, 1.82, dq, 3H, J=1.5, H-14.

^{13}C NMR: ppm

139.16 (C-1), 127.43 (C-2), 62.47 (C-3) 53.55 (C-5), 34.57 (C-6), 73.65 (C-7), 76.08 (C-8), 59.74 (C-9), 167.14 (C-10), 123.03 (C-11), 139.9 (C-12), 15.83 (C-13), 20.53 (C-14).

9-angelyl retronecine: (Name used in the literature, another name could be O^9 -angelylretronecine).



9-Angelylretronecine

Mass spectrum: m/z, (relative intensity)

237 (1.7), 193 (3.4), 154 (11.9), 138 (2.8), 137 (23.2), 136 (11.3), 94 (24.4), 93 (100), 83 (21), 80 (17.6), 69 (9), 67 (8.5).

^1H NMR: ppm, J (Hz)

5.81, br. s, 1H, H-2, 3.35, m, 1H, H-3A, 4.05, d, J=15.0, 1H, H-3B, 2.90, ddd, 1H, J=10.0, J=8.0, J=6.0, H-5A, 3.60, m, 1H, H-5B, 2.05, m, 2H, H-6, 4.46, m, 1H, H-7, 4.25, br. s, 1H, H-8, 4.77, t, 2H, J=13.5, H-9, 6.17, dq, 1H, J=7.5, J=1.4, H-12, 2.03, dq, 3H, J=7.4, J=1.4, H-13, 1.92, q, 3H, J=1.4, H-14.

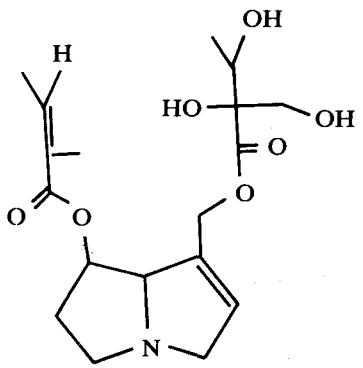
^{13}C NMR: ppm

133.17 (C-1), 127.4 (C-2), 61.92 (C-3), 53.86 (C-5), 35.64 (C-6), 70.60 (C-7), 78.52 (C-8), 60.45 (C-9), 167.1 (C-10), 126.69 (C-11), 140.70 (C-12), 15.97 (C-13), 20.41 (C-14).

Dehydroxytriangularine:

Mass spectrum: m/z (relative intensity)

369 (4.5), 338 (0.5), 269 (2.2), 252 (2.2), 237 (3.9), 220 (100), 119 (6.8), 138 (14.1), 137 (7.9), 136 (68.9), 121 (13.6), 120 (63.6), 119 (21.5), 106 (7.9), 95 (7.3), 94 (46.0), 93 (77.2), 80 (21.5).



Dihydroxytriangularine

^1H NMR: ppm, J (Hz)

5.86, m, 1H, H-2, 3.49, m, 1H, H-3A, 4.15, m, 1H, H-3B, 2.80, q, 1H, J=8, H-5A, 3.50, q, 1H, J=3.5, H-5B, 2.20, m, 2H, H-6, 5.52, m, 1H, H-7, 4.65, m, 1H, H-8, 4.81, s, 2H, H-9, 6.11, dq, 1H, J=7.0, J=1.5, H-12, 2.00, dq, 3H, J=7.0, J=1.5, H-13, 1.85, dq, 3H, J=1.5, J=1.5, H-14, 4.00, q, 1H, J=6.5, H-17, 1.20, d, 3H, J=7.0, H-18, 3.68, d, 2H, J=7.5, H-19.

^{13}C NMR: ppm

139.86 (C-1), 127.32 (C-2), 62.34 (C-3), 53.82 (C-5), 34.46 (C-6), 73.49 (C-7), 75.72 (C-8), 62.06 (C-9), 166.81 (C-10), 127.89 (C-11), 133.35 (C-12), 15.89 (C-13), 20.57 (C-14), 174.08 (C-15), 82.17 (C-16), 69.36 (C-17), 17.63 (C-18), 65.17 (C-19).

RESULTS AND DISCUSSION

The three identified alkaloids have been separated by DCCC and VLC after the preparation of the total alkaloids from the alcoholic extract. Vacuum liquid chromatography (VLC) has proven to be a quick and reliable chromatographic tool for the separation of pyrrolizidine alkaloids. Solvent mixtures of toluene: chloroform on alumina or silica gel could elute the different types of pyrrolizidine alkaloids separately. VLC of the total alkaloids of *A. orientalis* followed by preparative TLC afforded the pure alkaloids I, II and III.

The mass spectra showed that I and II have the molecular formula $C_{13}H_{19}NO_3$ and that they are isomers, while III has the molecular formula $C_{18}H_{27}NO_7$. The fragmentation patterns of the isomers I and II show that they are retronecine derivatives. However, a metastable fragmentation at m/z 137 is formed due to the cleavage of the side chain giving rise to an angelyl group ($C_5H_7O_2$) as the side chain. This indicates that one of the isomers is 7-angelyl retronecine and the other is 9-angelyl retronecine. The fragmention at m/z 237 in III show that the partial structure of 7- or 9-angelyl retronecine is present. Moreover, the difference between this framgment and the molecular ion indicates that III contains another ester of molecular formula $C_6H_{11}O_5$ suggesting that III is a retronecine diester, the second ester being 2-hydroxymethyl-2, 3 dihydroxybutanoic acid ester.

The ^1H NMR and the ^{13}C NMR data show that the ^1H values of 6.11 ppm for C-12 as well as the 2 methyl signals at C-13 and C-14 together with the ^{13}C values of 15.8 ppm for C-13, 20.53 ppm for C-14 as well as the presence of a carbonyl carbon at C-10, confirm the presence of angelic acid. Similar values can be found for II. The singlet for the two protons of III at 4.8 ppm for C-9 indicates that the alkaloid contains an acryclic diester. The structure of the acid which is esterified at C-7 has to be hydroxyangelic and that on C-9 has to be 2-hydroxymethyl 2, 3-dihydroxybutanoic acid. This is clear from the ^{13}C values for C-18 (17.63 ppm) and for C-19 (65.17 ppm) and from the coupling constants of the ^1H signal for C-17 (J=7Hz). Comparing these results with the published data (Roeder *et al*, 1984 and Roitman, 1988) showed that the data of 7-angelyl retronecine, 9-angelyl retronecine and dihydroxytriangularine coincide with the data obtained for I, II and III respectively. The alkaloid II, 9-angelylretronecine, has been isolated from *Alkanna* species for the first time.

REFERENCES

Afzal, M. and **M. Tofeeq, 1975.** 5, 8-Dihydroxy-2-(4-methylpent-3-enyl) 1, 4-naphthaquinone and its 2-(4-methyl crotonoloxy) pent-3-enyl analog (shikonin angelate) from *Alkanna hirsutissima.*, J. Chem. Soc., Perkin Trans 1(14): 1334-1335.

Bull, L.B., C.C. Culvenor and **A.T. Dick, 1968.** The pyrrolizidine Alkaloids; North Holland Publishing Co., Amsterdam.

Coll, J. and **B. Bowden, 1986.** The application of vacuum liquid chromatography to the separation of terpene mixtures., J. Nat. Prod., 49: 934-936.

Mansour, R.M. and **N.A.M. Saleh, 1986.** The flavonoids of *Alkanna orientalis*, J. Nat. Prod., 49: 355.

Otsuka, H., Y. Ogihara, and **S. Shibata, 1974.** Isolation of coclaurine from *Zizyphus jujuba* by droplet counter current chromatography, Phytochemistry, 13: 2016.

Papageorgiou, V.P., 1977. A new pigment of *Alkanna tinctoria* having naphthaquinone structure, Planta Med., 31: 390-394.

Papageorgiou, V.P., 1978. Wound healing properties of naphthaquinone pigments from *Alkanna tinctoria,* Experientia, 34: 1499-1501.

Pelletier, S.W., H.P. Chokshi and **H.K. Desai, 1986.** Separation of diterpenoid alkaloid mixtures using vacuum liquid chromatography., J. Nat. Prod. 49: 892-900.

Roeder, E., H. Wiedenfeld and **R. Schraut, 1984.** Pyrrolizidine alkaloids from *Alkanna tinctoria,* Phytochemistry, 23: 2725-2126.

Roitman, J.N., 1988. Longitubine and neolotifoline, new pyrrolizidine alkaloids from *Hackelia longitube,* Aust. J. Chem., 41: 1827-1833.

Taeckholm, V., 1974. Students' Flora of Egypt, published by Cairo University, printed by Cooperation printing Co., Beirut.