

PYRROLIZIDINE ALKALOIDS FROM *SENECIO AEGYPTIUS* L¹

By

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ABSTRACT

Investigation of the alkaloidal constituents of *Senecio aegyptius* L. resulted in the isolation and identification of senecionine, dehydrosenkirikine and probably an isomer of seneciphylline. TLC, HPLC and GLC showed the presence of integerrimine, seneciphylline, riddelliine and retrorsine.

INTRODUCTION

The genus *Senecio* (Compositae), have attracted a great deal of attention of several investigators during the last two decades, regarding their pyrrolizidine alkaloidal constituents. The interest in studying such class of alkaloids have increased dramatically in recent years, with the discovery that some of these alkaloids are carcinogenic and also the possibility that they may be involved in human liver diseases which often occur in populations using such plants in their food and herbal medicines (Smith and Culvenor, 1981; Lüthy *et al.*, 1983, 1984). Human exposure might also occur indirectly if residues of these alkaloids and/or their metabolites appear in tissues or milk products of animals, used for food (Deinzer *et al.*, 1977, 1982; Dickinson *et al.*, 1974, 1976).

Previous studies on *S. aegyptius* revealed the isolation of senecionine, otosenine, two unidentified alkaloids and choline in addition to the probable presence of riddelliine (Klasék *et al.*, 1968; Gharbo and Habib, 1969; Habib, 1981).

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1. This work represents part VI in the project entitled "Poisonous Plants Contaminating Edible Ones and Toxic Substances in Plant Foods", between the National Research Centre, Cairo, Egypt and the Food and Drug Administration, Washington, U.S.A.

EXPERIMENTAL

Plant Material

Senecio aegyptius L. was collected from Qena and Aswan provinces in June (during the flowering stage). The plant was kindly identified by Prof. Dr. M.N. El-Hadidi, Faculty of Science, Cairo University. The whole plant was air dried and ground into fine powder.

Apparatus and Techniques

Melting points were measured on Kofler microscope (uncorrected). GLC was carried out on Perkin-Elmer gas chromatograph with a gradient programmer using the following two conditions:

Condition I: Glass column, 6 ft. x 4 mm. i.d.; packed with 3% OV-101 on chromosorb W-AW (80-100 mesh); carrier gas, N₂; initial temp. 180°C; final temp. 240°C (rate 5°C/min.) using FID.

Condition II: Glass column, 6 ft. x 4 mm. i.d.; packed with 4% SE-30 on chromosorb Q (80-100 mesh); carrier gas, N₂; temp. 205°C (Isocaratic).

HPLC was carried out using Waters Associates Analytical Liquid Chromatograph Model 244, attached with a variable UV detector (Shofel model 450) used to monitor at 220 nm. and recorded on a Varian A-25 recorder. Column (Waters μ -Bondapack C₁₈, 30 cm x 4 mm. i.d.), developed with 61% methanol + 39% phosphate buffer (pH 6.4), as solvent; flow rate 1 ml/min.

TLC was applied on aluminium oxide G (Merck), developed with benzene-methanol-ammonia (90:10:0.5) and detection was carried out by spraying with Dragendorff's reagent.

MS were obtained by A.E.I., using MS-902 mass spectrometer.

Extraction of the Alkaloids (Tertiary bases and N-oxides):

About 500 gm. of the dried plant material were percolated with ethanol (3 x 1.5 l.). The extract was concentrated and the residue was taken up in dil. HCl, defatted with ether, reduced with zinc dust and filtered. The filtrate was made alkaline with ammonia and extracted with chloroform as previously described (Rizk *et al.*, 1983). Concentration of the chloroform extract gave a dark reddish brown gum (0.51 gm.; 0.1%).

TLC of the crude alkaloidal mixture revealed the presence of 7 alkaloidal spots. HPLC of the alkaloidal mixture showed the presence of 8 peaks (Table 1). GLC using condition I proved the presence of 12 peaks (Table 2).

Table 1
Retention Times of the Total Alkaloidal
Mixture (HPLC)

Peaks	R _t (minutes)	Alkaloids
1	2.4	
2	3.2	
3	4.6	
4	6.0	Senkirkine (?)
5	7.4	Riddelliine (?)
6	9.4	
7	11.7	Seneciophylline (?)
8	15.5	Senecionine and/or Integerrimine

Table 2
Retention Times of the Total Alkaloidal
Mixture (GLC, Condition I)

Peaks	R _t (minutes)	Alkaloids
1	4.8	
2	6.0	
3	8.4	
4	9.6	
5	10.4	
6	11.4	
7	12.2	
8	21.0	Senecionine
9	23.0	Integerrimine
10	27.0	Riddelliine and/or Retrorsine
11	34.0	Senkirkine
12	36.0	

Fractionation of the Alkaloids

Treatment of the alkaloidal mixture with ethanol, as previously described (Rizk *et al.*, 1983), afforded a white powder which gave, upon crystallization from methanol/chloroform a crystalline substance (A, 40 mg., m.p. 235-237°C). HPLC showed its identity with senecionine, while GLC revealed that it is mainly senecionine with a small amount of integerrimine. MS showed M^+ at m/e 335 and its fragmentation pattern was identical with both senecionine and integerrimine.

Column Chromatography of the Alkaloids

About 0.45 gm. of the alkaloidal mixture (after separation of the substance A), was chromatographed over 50 gm. aluminium oxide (neutral, grade I), packed in a glass column (65 cm x 0.5 cm. in benzene). Elution was accomplished by benzene, followed by benzene/methanol mixtures and collecting fractions (each 25 ml.).

Senecionine (I)

The residue obtained from fractions 39-41 (eluted with benzene-methanol 99:1), gave upon crystallization from methanol/chloroform a crystalline substance, m.p. 237-239°C (both alone and when admixed with authentic senecionine). TLC, GLC, HPLC and MS confirmed its identity as senecionine.

Alkaloidal Substance II (an isomer of seneciophylline)

The residue obtained from fractions 223-226 (eluted with benzene-methanol 90:10), was further purified by preparative TLC. MS showed M^+ at m/e 333 as well as ion peaks at 138, 136, 121, 120, 119 and 94. The alkaloid was found to possess different R_f (0.17) and R_t (GLC and HPLC) from those of seneciophylline.

Alkaloidal Substance III (Dehydrosenkirkine)

The residue obtained from fractions 46-48 (eluted with benzene-methanol 99:1; R_f 0,55) was further purified by preparative TLC. MS showed M^+ at m/e 363 and fragment ions at 335, 319, 168 (100), 150, 110 and 97 which are characteristic for otonecine type.

Alkaloidal Substance IV (Senkirkine)

The residue obtained from fractions 69-88 (eluted with benzene-methanol 99:1; R_f 0.48) was purified by preparative TLC. MS showed M^+ at m/e 365 and fragment ions at m/e 337(4), 321(6), 294(10), 266(18), 250(16), 222(16), 211(15), 168(46), 167(26), 153(57), 151(59), 149(100), 123(47), 122(40), 110(74), 83(58), 82(63), 81(60), 71(50), 70(59) and 69(39) which are in agreement with those of senkirkine (Culvenor *et al.*, 1976).

Alkaloidal Substance V

The alkaloid obtained from fractions 133-139 (eluted with benzene-methanol 99:1) was also purified by preparative TLC. MS of the alkaloid showed M^+ at m/e 168 and fragment ions at m/e 161, 150, 149, 110, 96 and 94 characteristic of the otonecine type.

RESULTS AND DISCUSSION

The method used for extraction of the alkaloids involved the extraction of the plant with ethanol. The concentrated extract was treated with dil. HCl and extracted with ether to remove chlorophyll and waxes. The acidic solution was treated with zinc dust for the reduction of the N-oxide alkaloids, then rendered alkaline with ammonia and the free alkaloids were extracted with chloroform (0.1%). Treatment of the crude alkaloidal mixture with absolute ethanol afforded a crystalline substance which was shown by TLC and HPLC to be senecionine (m.p. 237-239°C). However, GLC showed that it is mainly senecionine together with a small amount of integerrimine.

The application of column chromatographic technique of the mother liquor (after separation of substance A), followed by preparative TLC resulted in the isolation of senecionine, an isomer of seneciphylline, dehydrosenkirkine, senkirkine and an unidentified base of the otonecine type. The identity of senecionine and senkirkine was proved by TLC, GLC, HPLC and MS. The alkaloidal substance II was shown by MS to be a 12 membered macrocyclic diester of the retronecine type. Though the MS showed molecular ion at m/e 333 as well as fragment ions characteristic for seneciphylline, yet it was found to possess different R_f and R_t (GLC, HPLC) values from those of seneciphylline. It is probably an isomer of seneciphylline. The MS of the alkaloidal substance III showed molecular ion at m/e at 363. Moreover, the fragmentation pattern is in accordance with those of the pyrrolizidine alkaloids of the otonecine type. The alkaloid is probably dehydrosenkirkine and the acid moiety may be seneciphyllic acid. The MS of the alkaloidal substance V showed a molecular ion peak at m/e 168, in addition to the characteristic fragment ions of the otonecine type. In addition, GLC and HPLC confirmed the presence of the above alkaloids as well as the probable presence of riddelliine, retrorsine and seneciphylline.

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القلويدات البيروليزيدينية لنبات السينسيوإيجيبتياس

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أسفرت دراسة القلويدات البيروليزيدينية للنبات عن فصل والتعريف على السينوسيونين ، السنكركرين ، دي هيدروسنكركرين ومثابه للسينيسيفلين . كما أظهرت الدراسة باستخدام كروماتوجرافيا الطبقة الرقيقة وكروماتوجرافيا الغاز وكروماتوجرافيا السائل تحت الضغط المرتفع وجود قلويدات الانتجرمين ، السينيسيفلين ، الريدللين والرتورسين .