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Alkaloids of Genista acanthoclada DC.

Genista acanthoclada DC.'nın Alkaloitleri

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SUMMARY

In this research, six quinolizidine alkaloids were isolated from the aerial parts of *Genista acanthoclada* and their picrates were prepared. They were identified as sparteine, retamine, lupanine, anagyrine, N-methylcytisine and cytisine by TLC, melting points, IR spectra and elementary analysis. The total alkaloid content of aerial parts of *Genista acanthoclada* was found to be 0.12 % by using titrimetric method (1).

ÖZET

Bu çalışmada *Genista acanthoclada*'nın toprak üstü kısımlarından altı kinolizidin alkaloidi izole edilmiş ve pikrat tuzları hazırlanmıştır. Bu bileşikler, İTK, ergime dereceleri, IR spektrumları ve elementel analizleri ile spartein, retamin, anagirin, N-metilsitisin ve sitisin olarak teşhis edilmiştir. Titrimetrik yöntemle yapılan miktar tayininde *Genista acanthoclada*'nın toprak üstü kısımlarının total alkaloit miktarı % 0.12 olarak bulunmuştur.

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The presence of quinolizidine alkaloids in *Genista* species has been known for a long time. Although, there are a large number of publications dealing with the alkaloids of *Genista* species, there is only one paper dealing with *Genista acanthoclada* DC. (Fabaceae) (2).

In this study we isolated sparteine, retamine, lupanine, anagyrine, N-methylcytisine and cytisine from the aerial parts of *Genista acanthoclada* collected in Izmir (Turkey).

EXPERIMENTAL

The plant material was collected in May 1980 from Zeytinalan, Çeşme (Izmir) and identified by us. A voucher specimen, AEF No. 12701, is deposited in the Herbarium on the Faculty of Pharmacy, University of Ankara.

Elementary analysis were made with Perkin Elmer Model 240 Elemental Analyser. IR spectra were recorded in KBr discs with a Pye Unicam SP 1025 IR Spectrophotometer. Melting points were determined on a Buchi 510 Melting Point Apparatus and not corrected.

Extraction of total alkaloids

The dried and powdered aerial parts of *Genista acanthoclada* DC. (1 kg) were extracted in a Soxhlet apparatus with methanol. The extract was evaporated under vacuum to dryness. The residue was extracted with 5 % aqueous H_2SO_4. The combined aqueous acidic solutions were extracted with chloroform to remove the nonbasic substances, and this was discarded. The aqueous solution was then made basic (pH=12) with 33 % aqueous NaOH and extracted with chloroform. The combined chloroform extracts were dried with anhydrous Na_2SO_4, filtered and evaporated under vacuum to dryness, 1.14 g of a crude alkaloidal mixture was obtained. The crude alkaloidal mixture was dissolved in methanol and examined by thin-layer chromatography. For the TLC, silica gel GF_{254} and the developers S_1 (cyclohexane: diethylamine = 7:3), S_2 (cyclohexane: diethylamine = 9:1) and S_3 (chloroform: methanol: 25 % ammonium hydro-

xide = 85:15:1) were used. After drying for one hour at 120°C the chromatograms were examined under ultraviolet light 254 nm and then sprayed with Dragendorff reagent, six alkaloids appeared as orange-red spots with the following R_f values. S_1 (0.07, 0.30, 0.35., 0.61, 0.79, 0.93), S_2 (0.034, 0.07, 0.09, 0.24, 0.50, 0.83), S_3 (0.07, 0.18, 0.42, 0.61, 0.66, 0.69).

Isolation of the alkaloids from the crude extract

Preparative TLC was used for the separation of the crude alkaloidal extract. The crude alkaloidal mixture was dissolved in methanol and chromatographed on PLC plates of silica gel GF_{254}. F_1, F_2 and F_3 were obtained from the chromatogram with S_2 solvent system (Chromatogram-I). After the extraction of F_1, F_2 and F_3 from the chromatogram, the area between start and the spot with the $R_f = 0.24$ was eluted with chloroform: methanol (7:3) and evaporated under vacuum to dryness. The residue was dissolved in methanol and applied to PLC plates of silica gel GF_{254}. F_4, F_5 and F_6 were obtained from the chromatogram with S_3 solvent system (Chromatogram-II).

Quantitative analysis of total alkaloids

A crude alkaloidal mixture was obtained from the dried and powdered aerial parts of Genista acanthoclada (20 g) with the method mentioned above. The crude alkaloidal mixture was treated with excess of 0.01 N HC1 solution and back titration was made with 0.01 N NaOH solution using methyl red as indicator (1). This method was carried out in the same way for 5 times and average result was found as 0.12 %.

RESULTS and DISCUSSION

In this research, the alkaloids of the aerial parts of *Genista acanthoclada* were isolated and their structures were identified by IR spectroscopy and elementary analysis.

FAUGERAS and PARIS studied on *Genista acanthoclada* collected in Greece in 1967. They isolated retamine from stems and N-methylcytisine from flowers of this plant, and only identified lupanine, anagyrine and cytisine by gas chromatography. These workers mentioned that sparteine is not present in the aerial parts of the plant.

In this study, we have found the total alkaloid content as 0.12 % and isolated six quinolizidine alkaloids. After preparing their picrate salts we identified them as sparteine, retamine, lupanine, anagyrine, N-methylctisine and cytisine.

F_1 (Sparteine)

The upper band (R_f = 0.83) of the chromatogram-I was eluted with chloroform: methanol (7:3) and evaporated under vacuum. An oily residue remained. Its picrate was prepared. The picrate salt obtained was 68 mg, yellow needles, m.p. 208°C (3), R_f (S_1 0.93, S_2 0.83, S_3 0.07).

Analysis of the compound gave the following results: C, 46.87; H, 4.67; N, 16.10; 0, 32.36 %.

According to these results the molecular formula was calculated to be $C_{27}H_{32}N_8O_{14}$.

Elementary analysis, TLC, m.p. and IR spectra comparison of the salt with the standart sample showed that F_1 was sparteine. Figure-1 shows the IR spectra of the compound F_1 and sparteine dipicrate (prepared from Merck sparteine sulphate).

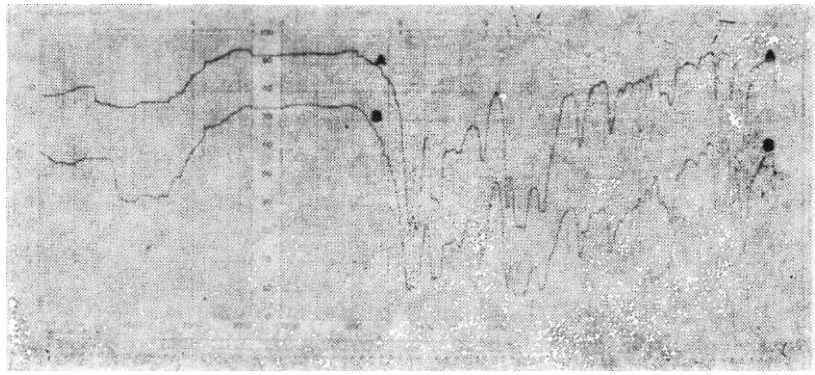

Figure-1. IR spectra of sparteine dipicrate and F,
A = Sparteine dipicrate, B = F_1

F_2 (Retamine)

The second band (R_f=0.50) was eluted with chloroform: methanol (7:3), concentrated under vacuum to a small volume and refri-

gerated for 48 hours. The yellow crystals which settled out were recrystallized from acetone and the crystals obtained were washed with cold acetone to give 108 mg colorless regular prisms with m.p. 164°C (4), R_f (S_1 0.79, S_2 0.50, S_3 0.18).

Analysis of the compound gave the following results: C, 71.90; H, 10.50; N, 11.18; 0, 6.42 %.

The molecular formula was calculated to be $C_{15}H_{26}N_2O$.

Elementary analysis, TLC, m.p. and IR spectra (5) comparison of the compound showed that F_2 was retamine.

The TLC, m.p. (168°C) and IR spectra (6) comparison of the dipicrate salt of the compound also proved its identity.

F_3 (Lupanine)

The third band (R_f=0.24) was eluted with chloroform: methanol (7:3) and concentrated under vacuum. Its picrate salt prepared was 82 mg, m.p. 179°C (7), R_f (S_1 0.61, S_2 0.24, S_3 0.69).

Analysis of the compound gave the following results: C, 52.75; H, 5.71; N, 14.60; 0, 26.94 %.

According to the results the molecular formula was calculated to be $C_{21}H_{27}N_3O_8$.

Elementary analysis, TLC, m.p. and IR spectra (6) comparison of the salt showed that F_3 was lupanine.

F_4 (Anagyrine)

The upper band (R_f =0.66) of the chromatogram-II was eluted with chloroform: methanol (7:3) and concentrated under vacuum to give a light yellow oily liquid. It was crystallized as picrate. Its picrate in needles was 96 mg, m.p. 243°C, R_f (S_1 0.35, S_2 0.09, S_3 0.66).

Analysis of the compound gave the following results: C, 53.18; H, 4.90; N, 14.92; 0, 27.00 %.

The molecular formula was calculated to be $C_{21}H_{23}N_5O_8$.

Elementary analysis, TLC, m.p. and IR spectra (8) comparison of the salt showed that F_4 was anagyrine.

F_5 (N-Methylcytisine)

The second band (R_f=0.61) was eluted with chloroform: methanol (7:3) and concentrated under vacuum to dryness. The residue was dissolved in methanol and precipitated as F_5 picrate. After crystallization, F_5 picrate was obtained 118 mg, long yellow needles, m.p. 229°C (9), R_f (S_1 0.30, S_2 0.07, S_3 0.61).

Analysis of the compound gave the following results: C, 50.01; H, 4.42; N, 16.14; O, 29.43 %.

According to these results the molecular formula was calculated to be $C_{18}H_{19}N_5O_8$.

Elementary analysis, TLC, m.p. and IR spectra (7) comparison of the salt showed that F_5 was N-methylcytisine.

F_6 (Cytisine)

The third band (R_f=0.42) was eluted with chloroform: methanol (7:3) and evaporated under vacuum to dryness. The residue was dissolved in methanol and precipitated as F_6 picrate. The picrate salt crystallized was 187 mg, m.p. 287 C, R_f (S_1 0.07, S_2 0.034, S_3 0.42).

Analysis of the compound gave the following results: C, 48.68; H, 4.12; N, 16.71; O, 30.49 %.

The molecular formula was calculated to be $C_{17}H_{17}N_5O_8$.

Elementary analysis, TLC, m.p. and IR spectra (10) comparison of the salt showed that F_6 was cytisine.

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