

Antifertility Screening and Phytochemical Investigation of *Ruellia prostrata* poir

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RUPELLIA prostrata poir (family : Acanthaceae) is a wild herbaceous plant. The juice of the leaves, boiled with little salt, is supposed to correct a depraved state of the humors on Malabar coast. It is also given with liquid copal as a remedy for gonorrhoea. The root of *R. suffruticosa* are given to cause abortion¹. Considering the medicinal importance of *Ruellia* species, the phytochemical investigation and antifertility screening of the plant were undertaken and the results reported in this note.

Experimental

The plant material (1.1 kg) was dried in shade, ground and extracted with boiling petroleum ether (b.p. 60–80°) for 24 h. After usual work up, the dark green neutral extract (6.2 g) was chromatographed over a column of silica gel (30-fold excess) which gave the following fractions :

Fraction A : Column on elution with petroleum ether and trituration from acetone yielded a white amorphous product (150 mg), m.p. 73–74° and homogeneous on tlc (CHCl₃ ; R_f, 0.92). Ir spectrum of this product indicated it to be a longchain aliphatic ester showing prominent peaks at 1715 and 1725 cm⁻¹. This fraction was then analysed by glc (alone and with added C₁₈-ester to serve as a standard for chainlength). It represented according to glc analysis, the homologous series of long-chain esters (C₄₂–C₆₀) with a maximum of C₅₄-ester (20.8%). Even-numbered members prevailed as usual^{2,3}. However, odd-numbered esters were also found in small quantities. The actual composition of fatty acids and alcohols in natural esters was not determined, but according to the results published^{4,5} earlier, it was supposed that single chromatographic peaks do not represent individual substances but mixtures of esters.

Fraction B : Further elution of the column with petroleum ether–benzene (3 : 1, v/v) gave lupeol, which upon crystallisation from methanol yielded shining crystals (75 mg), m.p. 211–12°. It was acetylated in Ac₂O–pyridine and the acetate was identified as lupeol acetate by glc (RRt, 1.93 ; OV-17 SCOT glass capillary column) and mass fragmentation pattern.

Fraction C : The next fraction on elution with petroleum ether–benzene (1 : 3, v/v) was composed of sterols, m.p. 126–28°. The fraction was acetylated in Ac₂O–pyridine. The glc analysis of this total acetylated sterol fraction indicated it to be a mixture of sitosterol, stigmasterol and campesterol.

Antifertility screening : The petroleum ether, ethanolic and aqueous extracts of *R. prostrata* poir were also screened for their possible antifertility effect on female albino rats by a method standardised by Khanna and Chaudhury⁶. In the present study, 40% activity was observed in the aqueous extract at a dose of 500 mg/kg and 20% in the petroleum ether and aqueous extracts at a dose of 100 mg/kg body weight ; while ethanolic extract was totally inactive.

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References

1. K. R. KIRTIKAR and B. D. BASU, "Indian Medicinal Plants", S. N. Basu, Allahabad, 1935, Vol. III, p. 1866.
2. S. HAMILTON and R. J. HAMILTON, *Top. Lipid Chem.*, 1972, 3, 199.
3. M. BEHARI, C. K. ANDHIWAL and M. STREIBL, *Collect. Czech. Chem. Commun.*, 1977, 42, 1385.
4. M. STREIBL, J. TIROUSOVA and K. STRANSKY, *Vette. Sclfen. Anstrachum.*, 1971, 73, 301.
5. M. STREIBL, K. KONECNY, A. TRKY, K. UBIK and M. PASLAR, *Collect. Czech. Chem. Commun.*, 1974, 34, 475.
6. U. KHANNA and R. R. CHAUDHURY, *Indian J. Med. Res.*, 1968, 56, 1575.

Free Amino Acids of *Argemone mexicana*

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Argemone mexicana Linn.¹ (Papaveraceae) is an annual herb with prickly leaves, bright yellow flowers and bristly capsules containing many seeds resembling black mustard seeds. It is an American plant which has run wild throughout India. The seed-oil and fresh yellow milky juice of the plant are used externally for the treatment^{1,2} of dropsy, jaundice, scabies, syphilitic ulcers, ophthalmia, respiratory disorders and constipation. Previous investigators reported the presence of a number of isoquinoline alkaloids²⁻¹⁰, flavonoids¹⁰⁻¹⁹, phenolics²⁰, sugars¹⁰ and fatty acids²¹⁻²³ in the plant,

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As amino acids are biogenetic precursors of several organic compounds, we attempted to find out the free amino acids from the plant. We chemically investigated the aerial parts of the young plant and isolated eleven amino acids among which three possess significant role of biogenetic precursors for alkaloids. In this communication, we report the isolation, identification and relative occurrence of these amino acids.

The aerial parts of the young plant (3 months old) *A. mexicana* L. were collected from Singur, West Bengal in March, 1984. The air-dried plant (500 g) were milled and extracted in a Soxhlet with petrol (b.p. 60–80°) and 90% ethanol successively for 48 h each. The alcoholic extract was concentrated and decolourised by refluxing with a little activated charcoal. The greenish filtrate was evaporated under reduced pressure to a gummy residue (15.5 g). The residue (1.5 g) was demineralised in the usual procedure^{24,25} by passing through a column of Amberlite IR 120(H). The column was washed with water till the eluate gave negative test with Molisch reagent for carbohydrates. The amino acids were then eluted with 4 N HCl. The elution was continued till a drop of eluate failed to give bluish violet colouration on a filter paper moistened with ninhydrin reagent (0.20% in acetone). The total eluate was concentrated under reduced pressure and paper chromatographed²⁶ in descending technique on Whatman no. 1 paper using solvent system n-BuOH—HOAc—H₂O (12 : 3 : 5) [Solvent-I.] and PhOH—H₂O—NH₄OH (5 : 1.25 : 0.9) [Solvent-II]. Amino acids were located on chromatogram by spraying ninhydrin reagent and heating the paper at 100° for a few minutes. *R_f* values of amino acids were measured and compared with those of authentic samples. The amino acids were identified as glycine, histidine, serine, proline, alanine, tyrosine, tryptophan and methionine by co-pc with authentic samples.

Another crude residue (1.5 g) was also desalted by passing through Amberlite IRA 400(OH) in the same manner as before. The amino acids were eluted with 1 N CH₃CO₂H. The eluate was concentrated under reduced pressure and paper-chromatographed in solvent-I. Three amino acids were found, identified as leucine, aspartic acid and glutamic acid by co-pc with authentic samples.

All these amino acids from their mixture were isolated as individual ninhydrin complex by preparative-pc²⁷ on Whatman (3 mm) using solvent-I as developing solvent and ninhydrin as spray reagent and heating the chromatogram at 100°. The coloured bands were mapped out by pencil and *R_f* of each coloured band was compared with that of authentic sample. Then each band was cut out in small strips and the strips were moistened with 75% ethanol (8 ml) in a petri dish for 0.5 h. The strips were then removed by thorough washing with 75% ethanol. The solution in the petri dish was transferred in a colorimeter tube and poured ninhydrin reagent (1 ml) to it and warmed the

mixture on a water-bath for 15 min. The coloured solution was cooled to room temperature, made up the volume to 10 ml with 75% ethanol and optical density (O.D.) was measured colorimetrically at 540 nm²⁸ by a Bausch & Lomb Spectronic 20 spectrophotometer. The O.D. of proline was measured at 440 nm²⁶. The amount of each amino acid was obtained by interpolation of its O.D. in the standard curve of authentic sample.

Thus, the relative occurrence of individual amino acid (in µg per g) of air-dried plant was tryptophan, 28.1; methionine, 13.2; proline, 12.8; alanine, 10.7; tyrosine, 8.8; histidine, 6.7; serine, 6.1; glycine, 5.1; leucine, 4.2; aspartic acid, 1.3 and glutamic acid, 0.8. Although phenylalanine was not found in the plant, tryptophan, tyrosine and methionine were found in appreciable amounts. Tyrosine and tryptophan are the biogenetic precursors of many alkaloids and methionine is the source of both O- and N-methyl groups of many alkaloids.

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References

1. "The Wealth of India. A Dictionary of Indian Raw Materials and Industrial Products", C.S.I.R., New Delhi, 1948, Vol. 1, p. 116.
2. B. C. BOSE, R. VIJAYVARGIYA, A. Q. SAIFI and S. K. SHARMA, *J. Pharma. Sci.*, 1963, **52**, 1172.
3. L. SLAVIKOVA and J. SLAVIK, *Collect. Czech. Chem. Commun.*, 1956, **21**, 211.
4. F. GIRAL and A. SOTELO, *Chem. Abstr.*, 1960, **54**, 3855.
5. B. K. RAZDAN and I. C. BHATTA, *Proc. Rajasthan Acad. Sci.*, 1963, **10**, 59 (*Chem. Abstr.*, 1965, **62**, 15071).
6. W. A. CHELOMBITKO, D. A. MURAJEVA and YU. EL SAWY, *Khm. Prer. Soedan*, 1971, **7**, 208.
7. D. K. SANTRA and A. N. SAOJI, *Curr. Sci.*, 1971, **40**, 548.
8. BUI THI YU and D. A. MURAJEVA, *Chem. Abstr.*, 1973, **79**, 15820.
9. BUI THI YU, *Chem. Abstr.*, 1974, **81**, 60863.
10. D. A. MURAJEVA and BUI THI YU, *Chem. Abstr.*, 1976, **84**, 147614.
11. K. HATSOVA and J. SLAVIK, *Collect. Czech. Chem. Commun.*, 1975, **40**, 1576.
12. W. DOEPKE, U. HESS and V. JIMENEZ, *Z. Chem.*, 1976, **16**, 54.
13. W. S. WOO, H. J. CHI and H. S. YUN, *Chem. Abstr.*, 1980, **92**, 107337.
14. S. F. HUSSAIN, S. NAKKADY, L. KHAN and M. SHAMMA, *Phytochemistry*, 1983, **22**, 319.
15. N. K. R. PATHAK, M. BISWAS, K. K. SETH, S. P. D. DWIVEDI and V. B. PANDEY, *Pharmazie*, 1985, **40**, 202.
16. W. RAHMAN and M. ILYAS, *C. R. Hebd. Seances Acad. Sci.*, 1961, **252**, 1974.
17. W. RAHMAN and M. ILYAS, *J. Org. Chem.*, 1962, **27**, 158.
18. M. KRISHNAMURTI, J. D. RAMANATHAN, T. R. SESHADRI and P. R. SHANKARAN, *Indian J. Chem.*, 1965, **3**, 270.
19. J. B. HARBORNE and C. A. WILLIAMS, *Phytochemistry*, 1983, **22**, 1520.
20. D. K. BHARDWAJ, M. S. BISHT, R. K. JAIN and A. MUNJAL, *Phytochemistry*, 1982, **21**, 2154.
21. V. V. S. MANT and G. LAKSHMINARAYAN, *Fette, Seifen, Anstrichm.*, 1972, **74**, 268.
22. S. B. MAHATO, N. P. SAHU, A. NARAYANSWAMI, R. N. CHAKRAVARTI and D. CHAKRAVARTI, *J. Indian Chem. Soc.*, 1975, **52**, 626.

23. F. D. GUNSTONE, J. A. HOLLIDAY and C. M. SCRIMGEOUR, *Chem. Phys. Lypads* 1977, 20, 331.
 24. E. LEDERER and M. LEDERER, "Chromatography", Elsevier, New York, 1957, p. 127.
 25. E. STAHL, "Thin-Layer Chromatography", 2nd. ed., Toppan, Tokyo, 1969, p. 737.
 26. D. T. PLUMMER, "An Introduction to Practical Biochemistry", 2nd ed., Tata McGraw-Hill, New Delhi, 1979, pp 83, 144.
 27. D. ABBOTT and R. S. ANDREWS, "An Introduction to Chromatography", Houghton Mifflin, Boston, 1965, p. 18.
 28. D. J. BHATT, A. J. BAXI and A. R. PARIKH, *J. Indian Chem. Soc.*, 1983, 60, 98.

Specific Separation of Chromium(III) from Mixture with numerous Metal Ions by Tlc on Silica Gel-G Layers in HCl and DMSO-HCl systems

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SILICA gel-G has been used extensively for the separation of organic and inorganic substances¹.

A number of non-aqueous systems have been used as eluants. A search of the literature reveals that no work has been reported on the specific separation of Cr^{III} from mixture with numerous metal ions on silica gel-G layers. The present work was therefore undertaken and a number of interesting separations achieved in pure DMSO and DMSO-HCl systems.

Experimental

A Toshniwal tlc apparatus for the preparation of silica gel-G layers on 20×3 cm glass plates was used. The plates were developed in glass jars (20×6 cm).

Silica gel-G (B.D.H., England) and DMSO (B.D.H., England) were used. All other chemicals were of AnalaR grade. Solutions (0.1 M) of chlorides, nitrates or sulphates of most of the cations were prepared in 0.1 M solutions of the corresponding acids. The solutions of sodium molybdate, sodium tungstate, sodium selenite and sodium tellurite were prepared in water. The solutions of Nb^V and Ta^V were prepared as reported earlier². Conventional spot-test reagents were used for detection purposes³.

Procedure: Silica gel-G plates (~0.15 mm thick) were prepared as usual. The cation solutions were spotted on the plates with thin glass capillaries. After drying the spots, plates were developed in the chosen solvent system by the ascending technique. The solvent was allowed to ascend 11 cm from the

starting line on the plate in all cases. R_f values were then measured as usual.

Solvent systems: The solvent systems used were: (i) 1 M HCl, (ii) DMSO, (iii) DMSO-1 M HCl (1 : 1), (iv) DMSO-1 M HCl (9 : 1), (v) DMSO-2 M HCl (1 : 1), (vi) DMSO-4 M HCl (1 : 9), (vii) DMSO-4 M HCl (1 : 1), (viii) DMSO-6 M HCl (1 : 9), (ix) DMSO-6 M HCl (3 : 7), (x) DMSO-6 M HCl (1 : 1), and (xi) DMSO-6 M HCl (9 : 1).

Systems studied: (i) Binary mixtures: Cr^{III} from Ag^I or Hg₂^{II} or Sn^{II} or Sn^{IV} or ZrO^{II} in 1 M HCl; Cr^{III} from Hg^{II} or Pd^{II} or VO^{II} or Pt^{IV} in pure DMSO; and Cr^{III} from Ag^I or Pb^{II} or Sb^{III} or Sn^{II} or Sn^{IV} or Y^{III} or ZrO^{II} or Th^{IV} or Ti^{IV} or In^{III} or Sm^{III} or Hg₂^{II} or K or Mn^{II} or Tl^I or Ba^{II} or Sr^{II} or WO₄²⁻ or TeO₄²⁻ or SeO₄²⁻ in DMSO-HCl systems in varying ratios. (ii) Ternary mixtures: Cr^{III}-Pb^{II}-Y^{III} or Ti^{IV} or TeO₄²⁻ or SeO₄²⁻ or Pt^{IV} in DMSO-1 M HCl (9 : 1); Cr^{III}-Ti^{IV}-Hg₂^{II} or Sn^{IV} or WO₄²⁻ or Nb^V or Ta^V in DMSO-4 M HCl (1 : 9); Cr^{III}-TeO₄²⁻-K or ZrO^{II} or WO₄²⁻ or Ba^{II} in DMSO-4 M HCl (9 : 1); Cr^{III}-MoO₄²⁻-WO₄²⁻ or Sn^{IV} or Mg^{II} or Nb^V in DMSO-4 M HCl (3 : 7); and Cr^{III}-ZrO^{II}-WO₄²⁻ or Ag^I or Nb^V in DMSO-6 M HCl (3 : 7).

Results and Discussion

The separations achieved are predictable and reproducible. This is a direct result of certain unique properties of DMSO, i.e. its high dielectric constant ($\epsilon=47.5$), its ability to solvate metal ions in preference to anions, and a specific solvating effect DMSO favours the formation of chloro complexes in an HCl medium⁴. The advantage of this work lies in the fact that we have been able to separate Cr^{III} ion from the mixtures containing larger amounts of other metal ions such as Ag^I, Hg₂^{II}, Sn^{II}, Sn^{IV}, ZrO^{II}, Pd^{II}, VO^{II}, Pt^{IV}, Pb^{II}, Sb^{III}, Y^{III}, Th^{IV}, Ti^{IV}, In^{III}, Sm^{III}, K, Mn^{II}, Tl^I, Ba^{II}, Sr^{II}, TeO₄²⁻, SeO₄²⁻ and WO₄²⁻. Out of these, the separation of Cr^{III} from Pb^{II}, Mn^{II}, VO^{II} and WO₄²⁻ is important in alloy analysis. In order to establish the utility of these separations, the synthetic alloy samples of Cr^{III} with Pb^{II}, Mn^{II}, VO^{II} and WO₄²⁻ were prepared by mixing various metallic solutions in certain ratios so that they correspond to the actual metallic proportions in the standard alloys, and then these samples were tried on silica gel-G plates and the metal ion separations were achieved.

The most interesting feature of the study is the increase in the R_f value of Cr^{III} from almost zero to nearly one by the addition of HCl to pure DMSO. This can be explained in terms of formation of chloro-complexes of the metal ion which are solvated by DMSO causing a higher R_f value. For most of the cations studied, there is no change in R_f values with the change in DMSO concentration. The high but constant R_f values for most of the ions are due to solvation of the cations or their chloro-complexes.

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