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SPIROSTANES OF *KALLSTROEMIA TRIBULOIDES*: IDENTIFICATION OF C-25 EPIMERS IN MIXTURE BY ^{13}C NMR SPECTROSCOPY

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Key Word Index—*Kallstroemia tribuloides*; Zygophyllaceae; spirostanes; epimeric mixture identification; ^{13}C NMR.

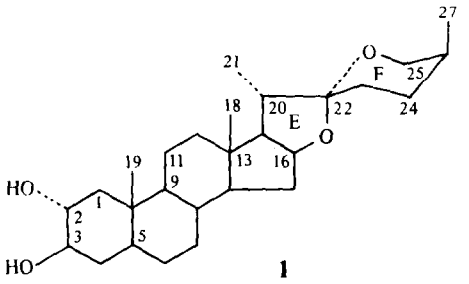
Abstract—Gitogenin and neogitogenin in a mixture were identified by ^{13}C NMR spectral analysis of the saponified fraction of the ethanolic extract of the above ground parts of *Kallstroemia tribuloides*. Tigogenin and stigmasterol were also identified.

INTRODUCTION

Our continued interest in finding a rich source of diosgenin, and other useful steroidal sapogenin bearing plants, led us to investigate *Kallstroemia tribuloides* Weight et Arn (Zygophyllaceae). This is an annual herb commonly known around northeastern Brazil as 'rabo de calango' and used externally in popular medicine for the treatment of boils. Diosgenin, the most important starting material for the partial synthesis of various hormonal steroids, is usually obtained from various *Dioscorea* species. However, it has also been encountered in other sources and has been reported [1] to be present in relatively high quantity in *Kallstroemia pubescens*, a tropical American species accidentally introduced in India.

The ethanolic extract of the aerial parts of *K. tribuloides*, upon acid hydrolysis, gave a crude sapogenin fraction which yielded an apparently homogeneous substance A in addition to tigogenin and stigmasterol. The homogeneity of substance A was ascertained by repeated crystallization which resulted in a material showing a single spot on TLC plates. We have earlier demonstrated [2, 3] the usefulness of ^{13}C NMR spectroscopy in identification of epimeric mixtures as well as of other complex mixtures of common natural products which otherwise would require extremely tedious separations. We wish to report here that based on ^{13}C NMR spectral data, the substance A has been identified as an intimate mixture of (25*R*)- and (25*S*)-spirostane epimers, gitogenin and neogitogenin (**1**).

The mass spectrum of this substance showed $[M]^+$ at *m/z* 432 corresponding to $C_{27}H_{44}O_4$. However, substance A did not have a sharp melting point. Acetylation of this substance gave a diacetate (IR, MS) which also showed a single spot on TLC while not showing a sharp melting point. The aforementioned data along with peaks at *m/z* 139 (100%) and 115 in the mass spectrum [4] indicated a dihydroxyspirostane structure. A stronger band at 920 cm^{-1} along with the usual 900 cm^{-1} in the IR spectrum suggested [5] the presence of a genin of the (25*S*)-series in substance A.



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The ^{13}C NMR spectrum of substance A showed 35 signals of which 27 are identical to those from gitogenin [6, 7]. The remaining 8 signals (Table 1) can only be assigned to C-20 to C-27 of rings E and F of (25*S*)-spirostanes by comparison with the corresponding shifts in sarsasapogenin [8] and jurubidin [9]. Therefore, substance A is a mixture of gitogenin and its (25*S*)-isomer, neogitogenin (**1**).

Table 1. ^{13}C NMR shifts (ppm) of C-20 to C-27 of substance A, gitogenin, sarsasapogenin and jurubidin

C	Substance A (25*R*)-	(25*S*)-	Gitogenin (25*R*)-	Sarsasapogenin (25*S*)-	Jurubidin (25*S*)-
20	41.9	42.4	41.5	42.1	42.2
21	14.4	14.4	14.0	14.3	14.3
22	109.6	110.0	109.0	109.5	109.7
23	31.5	27.3	31.0	27.1	27.1
24	28.9	25.9	28.6	25.8	25.8
25	30.5	26.2	30.0	26.0	26.0
26	67.1	65.0	66.7	65.0	65.1
27	17.2	16.1	17.7	16.1	16.1

EXPERIMENTAL

The ^{13}C NMR (20 MHz) spectra were run in $CDCl_3$/TMS. The complete proton noise decoupled spectra as well as APT spectra were run. Column chromatographic separations were done on glass columns using silica gel (Merck) as the stationary phase. TLC were performed with silica gel G (HF_{254}) plates using C_6H_6–EtOAc (43:7).

Extraction and separation. The above ground parts of *K. tribuloides* (800 g) was defatted (C_6H_{14}) and subsequently extracted with 90% EtOH for 30 hr in a Soxhlet. The concd extract was hydrolysed in the usual way with 10% H_2SO_4 for 2 hr. The crude sapogenin obtained in the usual way was dried and extracted with hexane. The hexane-soluble dark green sapogenin mixture was chromatographed on an alumina column and the benzene eluate gave a mixture from which tigogenin [10], mp 198–200°, acetate, mp 203–205° and stigmasterol, mp 168–169°; acetate, mp 142–144°, were subsequently isolated by PLC. The $CHCl_3$–MeOH eluate gave substance A which was crystallized several times from MeOH (0.8 g), mp 250–258°; diacetate, mp 228–232°.

REFERENCES

1. Chakravarti, R. N., Mahato, S. B., Sahu, N. P. and Pal, B. C. (1976) *J. Inst. Chemists (India)* **48**, 170.
2. Bhattacharyya, J. and De Carvalho, V. R. (1986) *Phytochemistry* **25**, 764.
3. Bhattacharyya, J. and Barros, C. B. (1986) *Phytochemistry* **25**, 274.
4. Rippergar, H., Budzikiewicz, H. and Schreiber, K. (1967) *Chem. Ber.* **100**, 1725.
5. Eddy, C. R., Wall, M. E. and Scott, M. K. (1953) *Anal. Chem.* **125**, 266.
6. VanAntwerp, C. L., Eggert, H., Meakins, G. D., Mines, J. O. and Djerassi, C. (1977) *J. Org. Chem.* **42**, 789.
7. Jain, D. C., (1987) *Phytochemistry* **26**, 1789.
8. Eggert, H. and Djerassi, C. (1975) *Tetrahedron Letters* 3635.
9. Redeglia, R., Adam, G. and Ripperger, H. (1977) *Tetrahedron Letters* 903.
10. Tschesche, R., Wulff, G. and Balle, G. (1962) *Tetrahedron* **18**, 959.

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CREPIDATIN, A BIBENZYL DERIVATIVE FROM THE ORCHID *DENDROBIUM CREPIDATUM*

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Key Word Index—*Dendrobium crepidatum*; Orchidaceae; crepidatin; bibenzyl derivative.

Abstract—Crepidatin, a new bibenzyl derivative, was isolated from the orchid *Dendrobium crepidatum*. Its structure was determined from spectral data.

INTRODUCTION

From a series of Indian orchids we reported [1–3, 5–25] earlier the isolation of a number of compounds which represent several structural types like bibenzyls [1, 2], phenanthrenes [3–9] (both monomeric and dimeric), phenanthropyrans [10] and pyrones [11], 9,10-dihydrophenanthrenes [12–14] (both monomeric and dimeric), 9,10-dihydrophenanthropyrans [15–20] and pyrones [11, 15–17, 21], triterpenoids [22, 23] and steroids [24, 25]. The orchid *Dendrobium crepidatum* was chemically investigated by Leander *et al.* [26, 27] who reported the isolation of several alkaloids from this plant. Further chemical investigation of this orchid has now afforded a new bibenzyl derivative, designated as crepidatin, which was shown to have the structure **1a** from the following evidence.

RESULTS AND DISCUSSION

Crepidatin, $C_{18}H_{22}O_5$ (M^+ 318), mp 99°, showed typical benzenoid UV absorptions, λ_{max} 211 and 279 nm ($\log\varepsilon$ 4.51 and 3.61). The phenolic nature of the compound was indicated by its characteristic colour reactions, alkali-induced bathochromic shifts of its UV maxima [λ_{max} 218 and 296 nm ($\log\varepsilon$ 4.34 and 3.59)] and by its IR band at 3342 cm^{-1}. Crepidatin formed a monoacetyl derivative, $C_{20}H_{24}O_6$ (M^+ 360), mp 64°, with acetic anhydride and pyridine, confirming the presence of a single phenolic hydroxyl group.

The ^1H NMR spectrum of crepidatin showed signals for a single phenolic hydroxyl proton at δ5.47 (disappeared on deuterium exchange), four aromatic methoxyl groups at δ3.81 (9H, *s*) and 3.83 (3H, *s*), four equivalent benzylic methylene protons at δ2.82 (4H, *s*), typical of the