

Note

(24S)-24-Ethyl-Cholestane-3 α ,5 α ,6 β -Triol from *Zanthoxylum Regnellianum*

Mara Silvia P. Arruda, Judysson Allan O. Brito and Alberto C. Arruda

*Departamento de Química, Universidade Federal do Pará, Campus Universitário,
66075-900 Belém - PA, Brazil*

Received: February 23, 1995; February 22, 1996

Foi estudado um espécime da espécie *Zanthoxylum regnellianum* (tamanqueira-amarela), de cujo caule isolaram-se 6 substâncias, sendo cinco de ocorrência comum no gênero, e a (24S)-24-etil-colestano-3 β ,5 α ,6 β -triol, inédita no reino vegetal. A identificação estrutural baseou-se na análise de RMN e comparação com algumas amostras autênticas.

The phytochemical examination of the stems of *Zanthoxylum regnellianum* resulted in the isolation of (24S)-24-ethyl-cholestane-3 β ,5 α ,6 β -triol and five common constituents of the *Zanthoxylum* species. The cholestane derivative has not been previously reported in plants. NMR analyses and TLC comparison with some authentic samples were carried out to confirm the identification of the known compounds.

Keywords: *Zanthoxylum regnellianum*, *Rutaceae*, (24S)-24-ethyl-cholestane-3 β ,5 α ,6 β -triol

Introduction

In continuation of our study of the *Zanthoxylum* genus^{1,2}, we have now dedicated special attention to the chemical investigation of the Amazon species. After extensive chromatography the hexanic extract of the stems of *Zanthoxylum regnellianum* Engl. (Rutaceae), collected in the state of Pará, was found to contain the cholestane derivative (**1**), appearing for the first time in plants, although previously isolated from marine sponges^{3,4}. (24S)-24-Ethyl-cholestane-3 β ,5 α ,6 β -triol is an amorphous solid, C₂₉H₅₂O₃, mp 255-256 °C, [a]_D²⁵ +12° (c = 0.002, MeOH). We also isolated the known compounds β -sitosterol, stigmasterol, the pentacyclic triterpene lupeol, the furoquinolinic alkaloid dictamnine, and the furocoumarin isopimpinellin.

These compounds were identified by comparing their spectral data (¹H-NMR, ¹³C-NMR, IR, UV, and MS) with those previously reported³⁻¹⁰ and/or with authentic samples on TLC (β -sitosterol, stigmasterol and lupeol).

Experimental

Equipment

IR spectra: Perkin-Elmer 727B; ¹H-NMR spectra: 200 MHz-Bruker AC-200, 300 MHz-Varian Gemini-300; ¹³C-

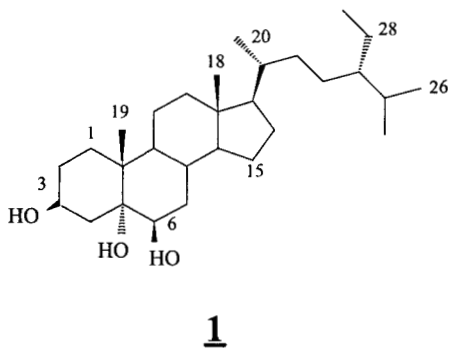
NMR spectra: 100 MHz-Bruker ARX-400, 75 MHz-Varian Gemini-300; Mass spectra: Finnigan model 1020 and Finnigan INCOS-XL; Optical rotation: digital polarimeter Perkin-Elmer model 341.

Plant material

Stems of *Zanthoxylum regnellianum* were collected in the Amazon Region, Brazil, in December 1991. The material was identified by N.O. Rosas, and a voucher specimen has been deposited in the Herbarium of IAN (Embrapa-CPATU)- (No. 164053), Pará, Brazil.

Extraction and isolation

The air-dried stems (4 kg) were extracted with hexane and CH₂Cl₂ at room temperature. The hexane extract (4.5 g) was chromatographed on a silica gel (120 g) column, eluted (each 50 mL) with hexane/CH₂Cl₂ mixtures (99:1, 97:3, 95:5, 9:1, 8:2, 1:1), CH₂Cl₂, CH₂Cl₂/EtOAc mixtures (99:1, 97:3, 95:5, 9:1, 8:2, 7:3), EtOAc and MeOH, and repeated column chromatography of different fractions yielded known compounds such as **1** (19 mg), as well as lupeol (30 mg), β -sitosterol (89 mg) and stigmasterol (63 mg). The CH₂Cl₂ extract (8.87 g) was applied on a silica gel (200 g) column, eluted (each 50 mL) with hexane/EtOAc mixtures (99:1, 97:3, 95:5, 9:1, 8:2, 7:3, 1:1),



EtOAc and MeOH, affording lupeol (250 mg), dictamnine (61 mg), isopimpinellin (50 mg), and β -sitosterol (370 mg).

(24S)-24-Ethyl-cholestane-3 β ,5 α ,6 β -triol (1)

An amorphous white solid, C₂₉H₅₂O₃; mp 255-256 °C; $[\alpha]_D^{25} +12^\circ$ (c = 0.002, MeOH); IR (ν_{\max} , film, cm⁻¹): 3430, 2960, 2880, 1460, 1385, 1045; ¹H-NMR (CDCl₃ + CD₃OD, 200 MHz, δ): 4.00 (1H, m, H-3), 3.46 (1H, bs, H-6), 2.02 (1H, dd, J 12.6 and 12.5 Hz, H_{ax}-4), 1.60 (1H, m, overlapped, H_{eq}-4), 1.16 (3H, s, 3H-19), 0.91 (3H, d, J 6.3 Hz, 3H-21), 0.84 (3H, t, J 7.3 Hz, 3H-29), 0.83 and 0.81 (3H each, d, both J 6.7 Hz, 3H-26 and 3H-27), 0.68 (3H, s, 3H-18); ¹³C-NMR (Pyridine-d₅, 75 MHz, δ): 32.54 (C-1), 33.35 (C-2), 67.42 (C-3), 42.87 (C-4), 75.96 (C-5), 76.34 (C-6), 35.77 (C-7), 31.26 (C-8), 45.99 (C-9), 39.20 (C-10), 21.82 (C-11), 40.74 (C-12), 43.13 (C-13), 56.66 (C-14), 24.68 (C-15), 28.62 (C-16), 56.66 (C-17), 12.44 (C-18), 17.24 (C-19), 36.56 (C-20), 19.06 (C-21), 34.34 (C-22), 26.64 (C-23), 46.18 (C-24), 29.60 (C-25), 19.30 (C-26), 19.99 (C-27), 23.48 (C-28), 12.20 (C-29); DEPT-¹³C NMR: is in agreement with this structure; EI-MS m/z (rel. int.): 448 (1) [M⁺], 430 (23), 412 (28), 398 (16), 290 (17), 271 (20), 262 (20), 248 (43), 247 (79), 244 (66), 230 (41), 229 (77), 211 (37), 161 (30), 149 (41), 135 (38), 123 (50), 107 (51), 95 (80), 81 (83), 69 (67), 55 (100).

Acknowledgments

The authors are grateful to the Universidade Federal de São Carlos, Departamento de Química, São Carlos - SP, Brazil, for optical rotations and ¹³C-NMR (100 MHz) spectra, and to the Universidade de São Paulo, Instituto de Física e Química de São Carlos - SP, Brazil for ¹H-NMR (200 MHz) spectra. Thanks are also due to the Universidade de São Paulo, Instituto de Química, São Paulo - SP, Brazil for EI-MS analysis, and to the Museu Paraense Emílio Goeldi, Belém, PA, Brazil for CG/MS analysis. One of the authors (J.A.O.B.) is grateful to the CNPq for the award of a Junior Research Fellowship.

References

1. Arruda, M.S.P.; Fernandes, J.B.; Vieira, P.C.; Silva, M.F.G.F.; Pirani, J.R. *Biochem. Syst. Ecol.* **1992**, *20*, 173.
2. Arruda, M.S.P.; Fernandes, J.B.; Silva, M.F.G.F.; Vieira, P.C.; Pirani, J.R. *Phytochemistry* **1992**, *31*, 3617.
3. Notaro, G.; Piccialli, V.; Sica, D. *J. Nat. Prod.* **1991**, *54*, 1570.
4. Das, B.; Srinivas, K.V.N.S. *J. Nat. Prod.* **1992**, *55*, 1310.
5. Itoh, T.; Tamura, T.; Matsumoto, T. *Phytochemistry* **1977**, *16*, 172.
6. Sangester, A.W.; Stuart, K.L. *Chem. Rev.* **1977**, *65*, 69.
7. Robertson, A.V. *Aust. J. Chem.* **1963**, *16*, 451.
8. Najjar, S.; Cordell, G.A.; Farnsworth, N.R. *Phytochemistry* **1975**, *14*, 2309.
9. Ashkenazy, D.; Friedman, J.; Kashman, Y. *Planta Med.* **1983**, *47*, 218.
10. Kokward, J.O.; Messana, L.; Galeffi, C.; Patamia, M.; Bettolo, G.B. *Planta Med.* **1983**, *47*, 251.