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Phytosterol and Flavanone from Roots of *Zanthoxylum budrunga*

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Phytochemical studies on the roots of *Zanthoxylum budrunga*, collected from Palaye, a village in Northern region of Goa, India, resulted in the isolation of two compounds. Their structures have been established as (3β, 4α, 5α, 24Z)-4-methyl stigmasta 7,24(28)-diene-3-ol (I) and 4′,5,7-trihydroxy-3′-methoxyflavanone (II) on the basis of spectroscopic techniques and chemical means. These compounds have been screened for their antimicrobial activity.

INTRODUCTION

Zanthoxylum budrunga is a lofty, deciduous tree up to 35 metres tall with a spreading crown, and bole of 4–6 metres. It is commonly distributed along the coastal lines of South India and Sub-Himalayan region in North India. The plant is reported to have antispasmolytic, antihelmintic and several therapeutic properties[1, 2]. The plant belonging to *Zanthoxylum* species is a source of various novel bio-active compounds[3]. The present paper describes the isolation and characterisation of two chemical constituents from the roots of the plant.

EXPERIMENTAL

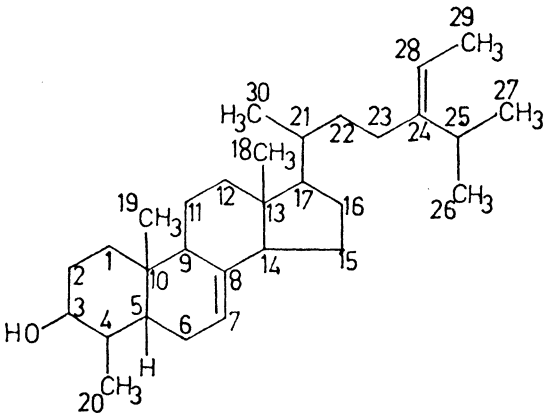
General experimental procedures

Melting points were determined in open capillaries and uncorrected. TLC was run on silica gel-G plates and spots were located by iodine vapours. UV spectra were recorded on a UV-160A, spectrophotometer Shimadzu. IR spectra were recorded on Perkin-Elmer FTIR-160 spectrophotometer. PMR spectra were recorded on Perkin-Elmer R-32 (90 MHz) and Bruker AMX (500 MHz) using TMS as an internal standard. Mass spectra were recorded on Jeol JMS-D300 spectrometer.

Extraction and Isolation

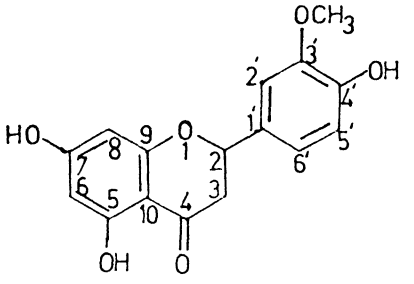
Compound I: The air-dried powdered roots (2 kg) were extracted with petroleum ether (60–80°C) and methanol in succession, in Soxhlet apparatus. Yellow coloured petroleum ether extract was evaporated to dryness under reduced pressure, to yield a thick viscous yellow coloured mass (2 g). TLC showed 5 spots in benzene and ethyl acetate (9:1). This yellow coloured mass was adsorbed on

a small portion of silica gel 80–120 mesh (20 g) with constant stirring until completely dried. The dried slurry was chromatographed over silica gel column, which was successively eluted with petroleum ether (60–80°C), benzene, chloroform and methanol in order of increasing polarity. Suitable combination of nonpolar and polar solvents were also tried to facilitate the elution. Petroleum ether and benzene (1 : 1) fraction was the major one. On evaporation of the solvent, yellowish white solid was obtained. On preparative TLC (benzene) followed by repeated crystallisation with methanol, it gave a white crystalline compound (1 g) m.p. 175–177°C. Compound gave positive test for sterol[4, 5] and formed an acetate m.p. 138–140°C.



(I) Δ_5-Sitosterol

Compound II: During the extraction of roots with methanol (successive), yellowish white amorphous compound deposited at the bottom of the flask; after recrystallisation with methanol, yellow coloured compound was obtained having m.p. 232–234°C. This compound showed single spot on TLC in ethyl acetate and methanol (9 : 1) and gave positive test for flavanone[6] and formed triacetate having mp 111–113°C.



(II) Homoeriodictyol

RESULTS AND DISCUSSION

Extraction of powdered roots of *Zanthoxylum budrunga* with petroleum ether (60–80°C) and methanol in succession, followed by detailed chemical analysis involving exhaustive TLC, column chromatography and spectral analysis resulted in isolation of two compounds. Compound I obtained from petroleum ether extract of roots was found to be α_1 sitosterol having m.p. 175–177°C.

Spectral analysis of Compound I. UV: λ_{max}^{MeOH} = 222 nm, IR: ν_{max}^{KBr} = 3150, 1490, 800 cm^{-1}. ^1H NMR: δ 0.7–0.9 (m, 18H, $9CH_2$), δ 1.0–1.2 (m, 21H, $7CH_3$), δ 1.3–1.5 (m, 10H, 10C—H), δ 4.5 (s, 1H, OH). EIMS m/z 426 (M^+)($C_{30}H_{50}O$), m/z 411, m/z 393, m/z 300, m/z 161, m/z 108, m/z 93. Acetylation of compound I with acetic anhydride gave a monoacetyl derivative m.p. 138–140°C. Structure proved by spectral and elemental analysis. IR: ν_{max}^{KBr} = 2990, 1490, 800 cm^{-1}. Elemental analysis: observed: C, 81.84; H, 11.13%; calculated for $C_{31}H_{52}O$: C, 81.87; H, 11.09%.

Based on the spectral and elemental evidences, the structure of compound I was elucidated as (3β, 4α, 5α, 24Z)-4-methyl stigmasta 7,24(28)-diene-3-ol.

Compound II obtained as amorphous powder from successive methanolic extract of roots has m.p. 232–234°C.

Spectral analysis of Compound II: UV: λ_{max}^{MeOH} = 228 nm, IR: ν_{max}^{KBr} = 3418, 2918, 1647, 1361, 804 cm^{-1}. ^1H NMR: δ 2.8 (d, 2H, CH_3), δ 4.8 (s, $3HOCH_3$), δ 5.1–5.6 (m, 3H, OH), δ 6.7–7.3 (m, 5H, Ar—H). ^{13}C NMR spectrum 78 (d, C-2), 47 (t, C-3), 202 (s, C-4), 167 (s, C-5), 102 (d, C-6), 172 (s, C-7), 101 (d, C-8), 168 (s, C-9), 109 (s, C-10), 137 (s, C-1′), 118 (d, C-2′), 154 (t, C-3′), 153 (s, C-4′), 120 (d, C-5′), 124 (d, C-6′), EIMS m/z 302 (M^+) ($C_{16}H_{14}O_6$), m/z 179, m/z 153, m/z 125. Acetylation of Compound (II) with dimethyl sulphate and acetic anhydride, gave triacetate derivative, m.p. 111–113°C, the structure of which was proved by spectral and elemental analysis. IR: ν_{max}^{KBr} = 3300, 2918, 1647, 1361, 804 cm^{-1}. [Observed: C, 60.97; H, 3.91%; calculated for $C_{19}H_{20}O_6$: C, 61.53; H, 4.80%]. On the basis of spectral data and elemntal analysis, the structure of Compound II was established as 4′,5,7-trihydroxy-3′-methoxylflavanone (homoeriodictyol).

Biological Activity

Antibacterial testing of plant is carried out by observing growth of various microorganisms when the extracts are placed in contact with them. The plant *Zanthoxylum budrunga* has already been proved to have antimicrobial activity. Hence α_1-sitosterol and homoeriodictyol were also screened for antibacterial activity[7].

Each extract was tested in triplicate with plate diffusion method. Negative control was carried out for dimethyl formamide. Honey which has been claimed to have antibacterial activity was kept as positive control. The average zone of emission is inclusive of the diameter of the cup 9 mm as given in Tables 1 and 2.

TABLE-1
ANTIBACTERIAL ACTIVITY OF THE α_1 SITOSTEROL

Micro-organism	Zone of inhibition (mm)		
	ALC	Water	Honey
S. aureus	18	14	21
B. subtilis	17.5	15	18
P. aeruginosa	21	18.3	23
E. coli	24	20	26

TABLE-2
ANTIBACTERIAL ACTIVITY OF THE HOMOERIODICTYOL

Micro-organism	Zone of inhibition (mm)		
	ALC	Water	Honey
S. aureus	14	12	21
B. subtilis	16	9.5	18
P. aeruginosa	20	16	23
E. coli	22	18	26

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REFERENCES

1. K.R. Kirtikar and B.D. Basu, Indian Medicinal Plants, 2nd Edn., Allahabad, India, Vol. 1, p. 464 (1918).
2. Wealth of India, CSIR, New Delhi, Vol. II, p. 17 (1976).
3. K.M. Nadkarni, Indian Materia Medica, Popular Book Depot, Vol. 1, p. 1302 (1954).
4. C. Liebermann, *Ber.*, **18**, 1803 (1885).
5. E. Salkowaski, Z.Physiol, Z. Physiol. Chem., **57**, 523 (1908).
6. T.A. Gaissaman, The Chemistry of Flavonoid Compounds, Oxford (1962).
7. The Antimicrobial Susceptibility Test: Principles and Practice, Le Febiger, Philadelphia, pp. 68, 76 (1978).

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