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Chemical Constituents of *Piper betle* Linn. (Piperaceae) roots

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Abstract: Column chromatography of the alcoholic extract of *Piper betle* roots furnished aristololactam A-II and a new phenyl propene, characterized as 4-allyl resorcinol, while the petroleum-ether extract yielded a diketosteroid, *viz.* stigmast-4-en-3,6-dione. All these compounds were characterized by spectroscopic means. Isolation of these compounds from this source is being reported here for the first time.

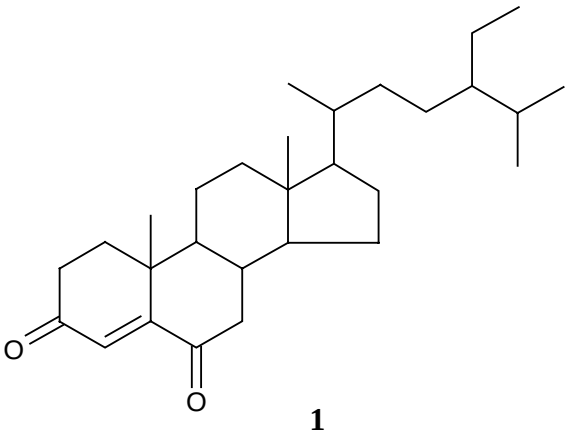
Keywords: *Piper betle*, Piperaceae, phenyl propene, diketosteroid, aristololactam A-II.

Introduction

Piper betle Linn. (Piperaceae) is a perennial dioecious creeper, probably native of Malaysia but cultivated in India for its leaves, used for chewing [1]. The leaf is carminative, aphrodisiac, tonic, laxative and improves appetite [2]. Leaves contained caryophyllene, cadinene, γ-lactone, allyl catechol, *p*-cymene and eugenol methyl ether in varying amounts [3,4]. The alcoholic extract of the leaf-stalk showed significant antifertility effects in both male and female rats [5-7]. Recently, cepharadione A, dotriacontanoic acid and tritriacontane were reported to be isolated from the petrol extract of leaves; piperine and piperlonguminine from petrol + dichloromethane extract of stems and β-sitosteryl palmitate from petrol + dichloromethane extract of root parts of *P. betle* [8]. In another study, the roots were found to contain 3β-acetyl ursolic acid and ursonic acid [9]. Traditionally, the roots of *P. betle* mixed with black pepper were said to be used as a contraceptive by women [10]. To elucidate and confirm the veracity of folklore antifertility claims, we report herein the further investigation of the constituents of *P. betle* roots.

Results and Discussion

Si gel column chromatography of the petroleum ether extract furnished stearic acid and β-sitosterol from petroleum ether-benzene eluates and a diketosteroid, *viz.* stigmast-4-en-3,6-dione (**1**) from the benzene eluates. Compound **1** gave a negative Liebermann-Burchard test for sterols. Its IR spectrum showed an absorption band at 1675 cm^{-1}, attributed to an α,β-unsaturated ketone. The ^{1}H-NMR spectrum showed a singlet at δ 6.17, attributed to the proton at the C-4 position flanked by two α,β-unsaturated ketones. This was consistent with literature values [11] and its identity was further confirmed by comparing it (UV, IR and TLC) with the oxidation product of β-sitosterol with Jones reagent. The subsequent alcoholic extract was further resolved into ethyl acetate and *n*-butanol extracts. Column chromatography of the ethyl acetate extract yielded 4-allyl resorcinol (**2**) from petroleum ether-benzene eluates and aristololactam A-II (**3**) from chloroform eluates. The identity of **3** was confirmed by comparing the reported ^{1}H-NMR data with those given in the literature [12] and also by direct comparison with an authentic sample. Isolation of compound **3** from the family Piperaceae has not been reported so far [8], although this basic skeleton has been isolated from Menispermaceae [13], Papaveraceae [14] and Aristolochiaceae [15].



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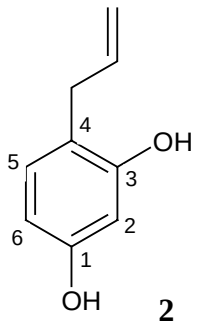

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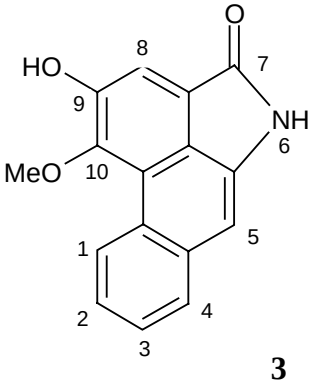

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Compound **2** possessed a characteristic phenolic odour and gave a bluish-black colouration with $FeCl_3$. Its ^{1}H-NMR spectrum showed several peaks at δ 3.25 (2H, d, J = 9 Hz), 5.05 (2H, m) and 5.91 (1H, m), characteristic of protons of an allyl moiety and other peaks at δ 6.61 (1H, dd, J = 9, 1.8 Hz), 6.70 (1H, d, J = 1.8 Hz) and 6.78 (1H, d, J = 9 Hz), indicative of protons of a 1,3,4-trisubstituted benzene. The mass spectrum of 4-allyl resorcinol is also consistent with the proposed structure **2**. This is the first naturally occurring phenyl propene of all the compounds isolated so far from *P. betle* roots.

Conclusions

The present study provides the first report on the occurrence of 4-allyl resorcinol, stigmast-4-en-3,6-dione and aristololactam A-II in *Piper* species. As a phenanthrene alkaloid has previously been shown to possess encouraging antifertility effects [16], the antifertility properties assigned to *Piper betle* roots in folklore may be due to this last active component or to the crude alcoholic extract.

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Experimental

General

Column chromatographic separation of compounds from the crude extracts was carried out using Si gel (60-120 mesh, Qualigens Fine Chemicals). Petroleum ether (Pet. ether) refers to the fraction of boiling range 60-80˚C. Melting points were determined in open capillaries in an electrical metal bath and are uncorrected. IR spectra were recorded using a RXI-FT-IR Perkin-Elmer spectrophotometer. ^{1}H-NMR (300 MHz) were recorded on a Bruker DPX 300 NMR spectrometer using TMS as internal standard and mass spectra on a JEOL JMS600 instrument.

Plant material

The roots of *Piper betle* were procured from M/s. United Chemical and Allied Products, Kolkata, India in the month of May 2002 and later authenticated at the Botanical Survey of India, Shibpur, West Bengal. The voucher specimen (DCT-2) was deposited in the laboratory of Department of Chemical Technology, Calcutta University for future reference.

Extraction and Isolation

Fresh tender roots of *P. betle* (ca. 2 kg) were shade-dried and powdered. The pulverised material was then exhaustively extracted in succession under reflux with pet. ether (2 x 5 L, 6 h each) and then alcohol (2 x 4.5 L, 8 h each). The pet. ether extract was concentrated *in vacuo* to yield a reddish-brown mass (15 g), subsequently subjected to column chromatography over Si gel (350 g). Elution with pet-ether and pet. ether-benzene (4:1) gave stearic acid (25 mg), m.p. 67-68°C. Elution with 1:1 pet. ether-benzene furnished β-sitosterol, while pure benzene eluates yielded stigmast-4-en-3,6-dione (**1**). The alcohol extract was concentrated *in vacuo* to a brown solid mass (32 g) and then suspended in distilled water (*ca* 200 mL). The aqueous slurry was then extracted successively with ethyl acetate (3 x 100 mL) and water saturated *n*-butanol (3 x 150 mL). The organic layers were dried over anhyd. Na_2SO_4 and the solvents distilled off *in vacuo* to yield 12 g of EtOAc and 17 g of *n*-butanol extracts, respectively. Column chromatography of the EtOAc extract furnished from the 1:1 C_6H_6-$CHCl_3$

eluates an impure brown oily matrix which on repeated chromatography followed by prep. TLC, yielded **2**. Subsequent elution with $CHCl_3$ yielded aristololactam A-II (**3**). The *n*-butanol extract did not give a positive test for saponins.

Stigmast-4-en-3,6-dione (**1**): Colourless flakes (28 mg) from 4:1 chloroform-alcohol; R_f 0.77 (benzene-ethyl acetate 7:3); m.p. 150-152°C (lit.[11] m.p. 156-158°C); UV λ_{max} (MeOH): 252 nm (log ε 4.26); IR ν_{max} (KBr) cm^{-1}: 2920, 2840, 1675 (α,β-unsaturated ketone), 1455, 1240, 1220; ^{1}H-NMR ($CDCl_3$) δ ppm: 0.72 (3H, s, Me-18); 0.74-0.95 (12H, m, 4 × Me); 1.16 (3H, s, Me-19) and 6.17 (1H, s, H-4); EIMS (70 eV) *m/z* (relative intensity, %): 426 ([M^+], 25), 412 (9), 411 (12), 408 (10), 398 (11), 384 (7), 285 (30), 275 (13), 257 (10), 243 (25), 229 (13.5), 189 (10), 175 (10), 149 (10), 137 (66), 133 (16), 97 (23), 69 (48), 55 (91).

4-Allyl resorcinol (**2**): Light brown viscous liquid (12 mg); R_f 0.74 (benzene-ethyl acetate 7:3); UV λ_{max} (MeOH) nm : 241 (log ε 3.47), 282 (3.66); IR ν_{max} (thin film) cm^{-1}: 3400-3250 (broad, –OH), 3060, 2950, 2890, 1630 (C=C), 1600, 1520, 1400 (aromatic, C=C skeletal in-plane vibration), 1355 (C–O stretching in phenol), 1285, 1200 (–OH bending in phenol), 1115, 970, 865, 815, 790, 760; ^{1}H-NMR ($CDCl_3$) δ ppm: 3.25 (2H, d, *J* = 9 Hz, –C*H*$_2$–CH=CH_2), 5.05 (2H, m, –CH=C*H*$_2$), 5.69 (2H, br s, 2 x ArO*H*), 5.91 (1H, m, –C*H*=CH_2), 6.61 (1H, dd, *J* = 9, 1.8 Hz, H-6), 6.70 (1H, d, *J* = 1.8 Hz, H-2) and 6.78 (1H, d, *J* = 9 Hz, H-5); EIMS *m/z* (relative intensity): 150 ([$M]^+$, 86), 123 (100), 95 (56), 67 (43). Unavailability of an authentic sample, however, precluded direct comparison.

Aristololactam A-II (**3**): Pale yellow amorphous solid from benzene (45 mg); R_f 0.43 (benzene- ethyl acetate 1:1); m.p. 260-262°C; UV: λ_{max} (MeOH) nm (log ε): 382 (3.66), 364 (3.68), 341 (3.80), 327 (3.79), 287 (4.27), 277 (4.33), 265 (4.25), 209 (4.54); (+ NaOAc): 385 (3.69), 367 (3.66), 307 (4.04), 288 (4.22), 278 (4.19), 264 (4.15), 212 (4.60); (+ 2N NaOH): 398 (4.03), 369 (3.99), 307 (4.36), 291 (4.37), 220 (4.68); IR ν_{max} (KBr) cm^{-1}: 3425, 1675 (C=O), 1620, 1500, 1455, 1415, 1365, 1325, 1290, 1270, 1230, 1195, 1120, 1060, 1035, 890, 875, 835, 735, 690; ^{1}H-NMR: (DMSO-d_6) δ ppm: 4.04 (3H, s, 10-OMe), 7.12 (1H, s, H-5), 7.54 (2H, m, H-2 and H-3), 7.76 (1H, s, H-8), 7.93 (1H, m, H-4), 9.27 (1H, m, H-1), 10.19 (1H, s, D_2O exchangeable, –OH) and 10.63 (1H, br s, D_2O exchangeable, –NH); EIMS (70 eV) *m/z* (relative intensity, %): 265 ([M^+], 100), 250 (55), 222 (28), 166 (34), 150 (7), 139 (21), 133 (8), 105 (7), 83 (8), 69 (12), 60 (9), 55 (13), 44 (18).

Oxidation of β-sitosterol with Jones reagent

To a solution of β-sitosterol (100 mg) in acetone (40 mL), Jones reagent [17] (0.3 mL) was added dropwise with vigorous stirring at room temperature. Upon complete addition of the reagent, the reaction mixture was stirred for an additional 15 min. Excess reagent was destroyed with isopropyl alcohol, water (50 mL) was added and the product was extracted with ethyl acetate (2 × 20 mL). The combined organic layers were washed with saturated sodium bicarbonate (3 ×10 mL), distilled water (2 ×10 mL) and then dried over anhydrous Na_2SO_4. Evaporation of the solvent left a semi-solid which was column chromatographed over Si gel. Elution of the column with 30-50% benzene in pet. ether yielded stigmast-4-en-3-one (20 mg), m.p. 95-97˚C, followed by **1** (22 mg), m.p. 153-155˚C; λ_{max} (MeOH): 250 nm (log ε 4.20); ν_{max} (KBr) 2930, 1675 cm^{-1}.

References

1. *The Wealth of India: Raw Materials*; Publications and Information Directorate, CSIR: New Delhi, **1969**; Vol. VIII, pp. 84-94.
2. Kirtikar, K.R.; Basu, B.D. *Indian Medicinal Plants*, *2nd ed*.; Bishen Singh Mahendra Pal Singh: Dehradun, India, **1998**; Vol. III, pp. 2131-2133.
3. Baslas, R.K.; Baslas, K.K. Chemistry of Indian essential oils - Part VIII. *Flavour Ind*. **1970**, *1*, 473-474; [*Chem. Abstr.* **1971**, *74*, 34538z].
4. Rastogi, R.P.; Mehrotra, B.N. *Compendium of Indian Medicinal Plants, Vol. 3*; Publications and Information Directorate: New Delhi, **1993**; pp. 502-503.
5. Sarkar, M.; Gangopadhyay, P.; Basak, B.; Chakrabarty, K.; Banerji, J.; Adhikary, P.; Chatterjee, A. The reversible antifertility effect of *Piper betle* Linn. on Swiss albino male mice. *Contraception* **2000**, *62*, 271-274.
6. Adhikary, P.; Banerji, J.; Chowdhury, D.; Das, A.K.; Deb, C.C.; Mukherjee, S.R.; Chatterjee, A. Antifertility effect of *Piper betle* Linn. extract on ovary and testis of albino rats. *Indian J. Exp. Biol.* **1989**, *27*, 868-870.
7. Adhikary, P.; Chowdhury, D.; Banerji, J.; Chatterjee, A. Antifertility effect of crude alcoholic extract of *Piper betle* stalk. *Ind. J. Physiol. Allied Sci.* **1998**, *52*, 22-27.
8. Parmar, V.S.; Jain, S.C.; Gupta, S.; Talwar, S.; Rajwanshi, V.K.; Kumar R.; Azim, A.; Malhotra, S.; Kumar, N.; Jain, R.; Sharma, N.K.; Tyagi, O.D.; Lawrie, S.J.; Errington, W.; Howarth, O.W.; Olsen, C.E.; Singh, S.K.; Wengel, J. Polyphenols and alkaloids from *Piper* species. *Phytochemistry* **1998**, *49*, 1069-1078.
9. Saeed, S. A.; Farnaz, S.; Simjee, R.U.; Malik, A. Triterpenes and β-sitosterol from *Piper betle*: isolation, antiplatelet and anti-inflammatory effects. *Biochem. Soc. Trans.* **1993**, *21*, 462S.
10. Khosa, R. L.; Singh, R.H. Betel root - an antifertility agent. *J. Res. Indian Med.* **1972**, *7*, 65-66.
11. Šolaja, B. A.; Milić, D.R.; Dosen-Mićović, L.I. Oxidation of steroidal 5-en-3β-ols with Jones reagent in ether. *Steroids* **1994**, *59*, 330-334.
12. Achari, B.; Chakrabarty, S.; Bandyopadhyay, S.; Pakrashi, S.C. A new 4,5-Dioxoaporphine and other constituents of *Aristolochia indica*. *Heterocycles* **1982**, *19*, 1203-1206.
13. Kunitomo, J.; Murakami, Y.; Oshikata, M.; *et al*. The alkaloids of *Stephania sasakii*: Structure of five new alkaloids. *Phytochemistry* **1980**, *19*, 2735-2739.
14. Castedo, L.; Suau, R.; Mourino, A. A revised structure for pontevedrine. *Tetrahedron Lett.* **1976**, *17*, 501-502.
15. Crohare, R.; Priestap, H.A.; Farina, M.; Cedola, M.; Ruveda, E.A. Aristololactams of *Aristolochia argentina*. *Phytochemistry* **1974**, *13*, 1957-1962.
16. Pakrashi, A.; Chakrabarty, B.; Das Gupta, A. Effect of the extracts from *Aristolochia indica* Linn. on interception in female mice. *Experientia* **1976**, *32*, 394-395.
17. Fieser, L. F.; Fieser, M. *Reagents for Organic Synthesis*; John Wiley and Sons: New York, **1967**; Vol. 1, pp. 142-143.

Sample availability: Samples of compounds **1** (5 mg) and **3** (8 mg) are available from the authors.