

Spermicidal Activity and Chemical Analysis of *Pentapanax leschenaultii*

G. Pant^{1,2}, M. S. Panwar¹, D. S. Negi¹, and M. S. M. Rawat¹

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Triterpenic glycosides have been reported to have spermicidal potential, furthermore "CONSAP" cream, with these constituents as the active compounds, has reached clinical trial as a male contraceptive (1). *Panax* and other araliaceous plants are rich sources of triterpenic glycosides. We recorded 100% immobilization of human spermatozoa by the ethanolic extract of the fresh leaves of *Pentapanax leschenaultii* (DC). Seem at the 2% level when assayed by the methods reported (2, 3). Since the plant has not been investigated, chemical analyses of the light petroleum- and *n*-butanol-soluble portions of the extract were carried out. The com-

pounds obtained were identified by the usual colour tests, physical data, IR, UV, MS; chemical degradation, and comparison with the authentic samples. The activity was found to be associated with a triterpenic glycoside from the butanol fraction.

The plant material, collected from Tungnath, U.P., was extracted with EtOH. The concentrated extract was partitioned into light petroleum: *n*-BuOH-, and H₂O-soluble portions. Column chromatography (silica gel, light petroleum-CHCl₃) gave the following compounds: hentriacontane, m.p. 66–68 °C (4), hentriacontanone, m.p. 82–83 °C (4), α -amyrin, m.p. 183–185 °C (4), β -sitosterol, m.p. 134–136 °C (5), and oleanolic acid, m.p. 304–306 °C (6).

The *n*-BuOH portion on CC (silica gel, CHCl₃-MeOH) gave the following compounds: β -sitosterol- β -D-glucoside, m.p. 134–136 °C (5), quercetin, m.p. > 300 °C (7), quercetin-3-*O*-rhamnoside, m.p. 180–184 °C

(7), and a glycoside which, on acidic hydrolysis, gave a triterpenic aglycone and glucose and arabinose. Its further characterization is in progress.

Acknowledgements

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¹ Department of Chemistry, Garhwal University, Srinagar Garhwal, 246 174, India.

² Address for correspondence.

Methyl Ester of *para*-Coumaric Acid: Antifungal Principle of the Rhizome of *Costus speciosus*

B. M. Ratnayake Bandara¹, Chandralal M. Hewage¹, Veranja Karunaratne^{1,3}, and N. K. B. Adikaram²

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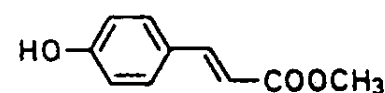
Costus speciosus (Koen) Sm. (Zingiberaceae) is a herbaceous plant growing in India, Malaya, Philippines, and Sri Lanka. The rhizome of this plant is used for the treatment of catarrhal fevers, worms, dyspepsia, and skin diseases (1).

An antifungal constituent of the rhizome has been identified as the methyl ester of *p*-coumaric acid.

The air-dried and powdered rhizome (84 g) of *C. speciosus* was extracted with methanol under reflux conditions. This extract displayed a single zone of inhibition at R_f = 0.3 (eluant chloroform) in the *Cladosporium*-TLC-bioassay (2). A part of the extract (8 g) was chromatographed on silica gel. The column fraction eluted with 60% chloroform in petroleum ether (b.p. 40–60 °C) exhibited antifungal activity. Further purification of this

fraction by flash chromatography on silica gel yielded the antifungal compound as a white crystalline solid (21 mg), m.p. 133–134 °C, C₁₀H₁₀O₃. From the spectral data and mass spectral fragments at *m/z* 178 (M, 72%), 147 (M-OCH₃, 100%), and 119 (M-CO₂CH₃, 43%), the compound was identified as methyl 3-(4-hydroxyphenyl)-2*E*-propenoate (1).

Both dichloromethane and ethanol extracts of the rhizome contained the active compound 1 (TLC, *Cladosporium*-TLC-bioassay). Thus the possibility that 1 is an artefact formed during the methanol extraction was eliminated. Inoculation of fresh rhizome with the fungus *Gloeosporium mangiferae* did not improve the yield of the active compound 1. This suggests that 1 is a preformed antifungal compound present in the rhizome and not a phytoalexin.



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¹ Department of Chemistry, University of Peradeniya, Peradeniya, Sri Lanka.

² Department of Botany, University of Peradeniya, Peradeniya, Sri Lanka.

³ Address for correspondence.

The compound **1** inhibited the growth of the following fungi: *Aspergillus niger*, *Cladosporium cladosporioides*, *Colletotrichum gloeosporioides*, *Curvularia* sp., and *Penicillium* sp.

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A Germacrene Alcohol from Fresh Aerial Parts of *Echinacea purpurea*

Rudolf Bauer¹, Peter Remiger¹, Victor Wray², and Hildebert Wagner^{1,3}

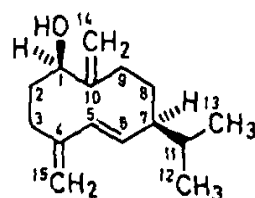
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During our search for the immunostimulatory principles of *Echinacea* species we recently described some new constituents of the aerial parts and of the roots of *E. purpurea* (L.) Moench, *E. angustifolia* DC., and *E. pallida* (Nutt.) Nutt. (1, 2, 3, 4). We now report on the isolation and structure elucidation of a sesquiterpene alcohol from fresh aerial parts of the same *Echinacea* species.

In freshly harvested aerial parts of *E. purpurea*, *E. angustifolia*, and *E. pallida* we detected, in addition to the alkylamides formerly found in lipophilic fractions of dried plant material (1), a further main constituent. The compound was isolated by alcoholic extraction of fresh aerial parts of *Echinacea purpurea* at room temperature and subsequent fractionation by column chromatography on silica gel. Purification was achieved by semi-preparative HPLC on reversed phase material (RP-18). The UV spectrum ($\lambda_{\max} = 239$ nm; $\epsilon = 18300$) of the compound indicated a system of two conjugated double bonds. This was confirmed by bands at 1640 and 1610 cm^{-1} in the IR spectrum, which also clearly showed the presence of an OH group (3350 cm^{-1}). The mass spectrum ($M^+ = 220$) with the molecular formula $\text{C}_{15}\text{H}_{24}\text{O}$ made a sesquiterpenoid structure very likely. The $^1\text{H-NMR}$ spectrum

showed four characteristic singlets in the olefinic area indicating two exocyclic double bonds. In the methylene region ($\delta = 1.2$ – 2.8 ppm) we observed a very complex pattern of signals resulting from the magnetic inequivalence of geminal protons in a cyclic system. The data of the COSY NMR, the $^{13}\text{C-NMR}$ spectrum, the $^1\text{H-}^{13}\text{C}$ correlated NMR spectrum, and nOe experiments revealed the presence of a germacrene alcohol structure. From the coupling constants in the $^1\text{H-NMR}$ spectrum the configuration of the endocyclic double bond was assigned as *trans* ($J = 16$ Hz). The optical rotation was determined as $[\alpha]_D^{20} = -189.6^\circ$ (c 0.48; hexane). According to data published by Fattorusso et al. (5) the stereochemistry at C-1 and C-7 could be assigned as 1*S*, 7*R*. Therefore both the hydroxy and the isopropyl group are in a β -position, leading to the structure of a germacra-4 (15), 5*E*, 10 (14)-trien-1 β -ol. This compound has previously been isolated from *Inula cuspidata* C. B. Clarke (6). A closely related compound, the sesquiterpene germacrene D, has recently been found in the aerial parts of *E. purpurea* by Bohlmann and Hoffmann (7).

The fact that we could not find the germacrene alcohol in any dried sample of *Echinacea* herbs might be due to its high volatility.



Materials and Methods

950 g of fresh cut aerial parts of *Echinacea purpurea* (L.) Moench, cultivated in the Institute of Pharmaceutical Biology, Munich (voucher specimen deposited) were extracted with 86% EtOH at room temperature for ten days. Chlorophyll and phenolic compounds were precipitated by adding a 5% aqueous solution of Pb-acetate. The remaining extract was fractionated by column chromatography on silica gel (30×5 cm, particle size < 0.063 mm) with *n*-hexane-EtOAc 4:1 as eluent. Final semi-preparative HPLC (LiChrosorb RP-18, 7 μm , 250×10 mm, 55% acetonitrile as eluent) afforded 40 mg of the pure compound as a colourless oil.

UV spectra were recorded online by diode array detection (Hewlett Packard 1040A) in acetonitrile-water mixtures. IR spectra: Beckmann Acculab 1 (KBr). Mass spectra: Kratos MS 80 RFA. NMR spectra: Bruker AM-300.

Germacra-4 (15), 5E, 10 (14)-trien-1 β -ol: Colourless oil; $[\alpha]_D^{20} = -189.6^\circ$ (c 0.48, hexane); UV λ_{\max} nm: 239 ($\epsilon = 18300$); IR ν_{\max} cm^{-1} : 3350 (OH); 3080, 885 ($-\text{C}=\text{CH}_2$); 2950 (CH); 1640, 1610 ($-\text{C}=\text{C}=\text{C}-$); 1380, 1360 [$-\text{CH}(\text{CH}_3)_2$]; 970 ($-\text{C}=\text{C}-$); EI-MS m/z (rel. int.): 220 (26) $[\text{M}]^+$, 205 (32) $[\text{M}-\text{CH}_3]^+$, 202 (55) $[\text{M}-\text{H}_2\text{O}]^+$, 192 (20), 187 (34) $[\text{M}-\text{CH}_3-\text{H}_2\text{O}]^+$, 177 (62) $[\text{M}-\text{C}_3\text{H}_7]^+$, 159 (67) $[\text{M}-\text{C}_3\text{H}_7-\text{H}_2\text{O}]^+$, 149 (63), 135 (71), 122 (73), 109 (86), 83 (94), 69 (92), 55 (99), 43 (100); $^1\text{H-NMR}$ (300 MHz, CDCl_3 , TMS int. standard, δ values in ppm): 5.98 (1H, d, $J = 16$ Hz, H-5), 5.41 (1H, dd, $J = 16$ Hz, 10 Hz, H-6), 5.24 (1H, s, H-15a), 4.97 (1H, s, H-15b), 4.90 (1H, s, H-14a), 4.82 (1H, s, H-14b), 3.74 (1H, dd, $J = 12$ Hz, 4 Hz, H-1), 2.59 (1H, ddt, $J = 12$ Hz, 7 Hz, 2 Hz; H-9a), 2.41 (1H, dt, $J = 5$ Hz, 13 Hz; H-3a), 2.16 (1H, ddd, $J = 13$ Hz, 6 Hz, 3 Hz; H-3b), 2.03 (2H, m, H-2a, H-8a), 1.78 (1H, m, H-7), 1.66 (3H, m, H-2b, H-9b, H-8b), 1.47 (1H, dq, $J = 7$ Hz, 13 Hz, H-11), 0.88 (3H, d, $J = 7$ Hz; H-12), 0.79 (3H, d, $J = 7$ Hz, H-13). $^{13}\text{C-NMR}$ (75 MHz; CDCl_3 ; TMS; δ values in ppm): 153.5 (s, C-4), 146.8 (s, C-10), 137.9 (d, C-6), 129.7 (d, C-5), 112.8 (t, C-15), 110.5 (t, C-14), 75.9 (d, C-1), 52.5 (d, C-7), 36.2 (2 \times t, C-8, C-2), 34.5 (t, C-9), 31.8 (d, C-11), 30.0 (t, C-3), 20.7 (q, C-12), 20.5 (q, C-13).

¹ Institut für Pharmazeutische Biologie der Universität München, Karlstr. 29, D-8000 München 2, Federal Republic of Germany.

² Gesellschaft für Biotechnologische Forschung mbH, Mascheroder Weg 1, D-3300 Braunschweig, Federal Republic of Germany.

³ Address for correspondence.

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Nor-Neolignans from *Krameria grayi*¹

Xorge A. Dominguez², Julia Verde Star², Carlos Rombold³, Diego Valdez³, Sergio Moreno³, Hans Achenbach^{4,5}, and Johann Groß⁴

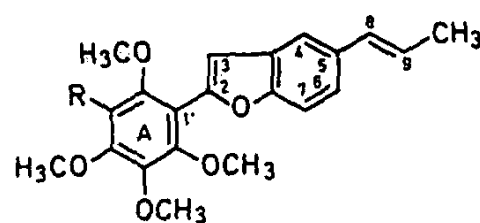
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Among the Yaqui indians from Sonora (North Western Mexico) it is an old tradition to drink daily several glasses of an aqueous extract of "Cosahui" to keep themselves healthy and fit (2,3). "Cosahui" is the indian name for *Krameria sonorae*, Britt. (Krameriaceae); but frequently this drink is prepared erroneously from *K. grayi*, Rose & Painter. In the course of time, this habit has been taken over by other people living in Sonora, who now use "Cosahui" even to cure stomach ulcers.

Recently it was reported that a neolignan from *Zizyphus jujuba* Mill (Rhamnaceae) can induce endogenous prostaglandin I₂ release (4). Since structurally related neolignans have been found in *Krameria sonorae* (5) these constituents might be responsible for the health effects of "Cosahui". Therefore, a screening of *K. grayi* for the presence of the same or structurally related neolignans was of interest.

Repeated chromatography of the hexane extract of aerial parts from *K. grayi* yielded as the main fraction a mixture of phytosterols. In contrast to other

Krameriaceae recently investigated (1, 5, 6), only a considerably minor fraction contained phenylpropanoids. The two "major" components of this fraction were isolated and their structures established to be ramosissin [1; 2-(2,3,4,5,6-pentamethoxyphenyl)-5-(*E*)-propenylbenzofuran] and 2-(2,3,4,6-tetramethoxyphenyl)-5-(*E*)-propenylbenzofuran (2).



1 R = OCH₃ ramosissin
2 R = H

Both nor-neolignans have been recently described for the first time as characteristic constituents of *K. ramosissima*, Gray (1). The result shows, that the lignans very unlikely are responsible for the health effect of "Cosahui"; it furthermore demonstrates, that, from a chemotaxonomic point of view, *K. grayi* might be more closely related to *K. ramosissima* than to *K. sonorae*.

Materials and Methods

Plant material

Plants were collected around the Jaqui Valley, Sonora (Mexico), in April 1986; botanical identification was done by D. Valdez, CIDESON, Hermosillo (Mexico); a voucher specimen is kept at the ITESM herbarium (Reg. No. 8130) in Monterrey (Mexico).

Extraction and chromatography

Aerial parts of dried plant material (80 g) were extracted successively with hexane and MeOH to yield 3.9 g of a hexane extract and 10.3 g of a MeOH extract. 300 mg of the hexane extract were chromatographed on silica gel (Macherey-Nagel 81538) using cyclohexane-EtOAc: a mixture of phytosterols (ca. 200 mg, not further investigated) was eluted first and a later fraction contained the phenylpropanoid compounds. Further separation and purification of this fraction on silica gel columns using CHCl₃-MeOH and CHCl₃ yielded 1 (1.5 mg) and 2 (1 mg).

Identification of 1 and 2

Identification is based on the chromatographic and spectroscopic properties of the isolated compounds and their comparison with authentic substances (1).

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¹ Part 4 in the series "Studies on Krameriaceae"; for part 3, see Ref. (1).

² Departamento de Química, Instituto Tecnológico y de Estudios Superiores de Monterrey (= ITESM), Monterrey, N.L. 64849, Mexico.

³ CIDESON, Hermosillo, Sonora, Mexico.

⁴ Institute of Pharmacy and Food Chemistry, Department of Pharmaceutical Chemistry, Universität Erlangen, D-8520 Erlangen, Federal Republic of Germany.

⁵ Address for correspondence.

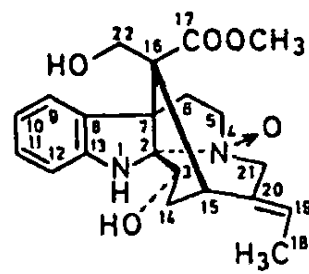
N_b -Demethylechitamine N -Oxide from Roots of *Winchia calophylla*

Chen Wei Ming^{1,2}, Zhang Pei Ling¹, and G. Rücker^{2,3}

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Winchia calophylla A. DC. (syn. *Alstonia pachycarra* Merrill et Chun) (Apocynaceae) (in Chinese "Pen Jin Shu"), natively abundant in the Yunnan province and Hainan island (China), represents one of two species belonging to the genus *Winchia* (1). The plant is used in the traditional medicine to treat cough, asthma, and acute and chronic bronchitis (2). From the ethanol extract of the roots of *W. calophylla* by the usual alkaloid isolation procedure, a mixture of compounds was isolated, from which four major components could be separated by column chromatography. Three of them were identified by their physical constants, spectral data, and acetylation products as N_b -demethylechitamine (1) (3), echitamine chloride (3), and loganine (4).

A further alkaloid (2), $C_{21}H_{21}H_2O_5$, shows UV and IR spectral data similar to those of 1. The relative molecular mass however, is 16 amu higher. The intensive fragmentation (97%) of $M - 16$ at m/z 370 is typical for N -oxides. In the ^{13}C -NMR spectrum, C-5 and C-21 bound to N_b , show large downfield shifts and C-6 and C-20 upfield shifts in relation to 1. These data indicate the structure of N_b -demethylechitamine N -oxide (2) which was confirmed by preparation of 2 from 1 by hydrogen peroxide oxidation.



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¹ Institute of Materia Medica, Chinese Academy of Medical Sciences, 1 Xian Nong Tan Street, Beijing, Peoples' Republic of China.

² Pharmazeutisches Institut, University of Bonn, Kreuzbergweg 26, D-5300 Bonn 1, Federal Republic of Germany.

³ Address for correspondence.

Table 1. 1H - and ^{13}C -NMR data of 1 and 2 ($CDCl_3$, TMS).

H/C	1H -NMR, δ [ppm]		^{13}C -NMR, δ [ppm]	
	1	2	1	2
2	—	—	95.1	97.5
3	4.10 (br. d)	4.53 (dd)	68.3	69.8
5	2.58 (dd)	3.3–4.9 (m)	53.8	65.0
5'	3.21 (dt)		—	—
6	1.80 (dd)	2.12 (dd)	46.4	39.6
6'	1.93 (dt)	2.35 (dt)	—	—
7	—	—	55.9	55.0
8	—	—	131.1	131.0
9	7.58 (d)	7.70 (d)	127.0	127.2
10	6.46 (dt)	6.82 (dt)	116.4	118.6
11	6.84 (dt)	7.10 (dt)	126.8	128.4
12	6.35 (d)	6.80 (d)	108.0	108.9
13	—	—	149.1	146.9
14	1.36 (ddd)	1.7 (dd)	32.0	32.7
14'	2.46 (ddd)	2.74 (ddd)	—	—
15	3.68	3.86	34.5	34.9
16	—	—	60.5	57.7
17	—	—	174.4 ^a	172.8
18	1.69 (dd)	1.87 (dd)	14.3	14.1
19	5.23 (q)	5.78 (q)	125.4	126.0
20	—	—	140.6	129.2
21	4.10 (d)	4.10 (d)	57.0	73.0
21'	2.80 (d)	3.3–4.9 (m)	—	—
22	3.14 (dd)	3.28 (d)	65.5	65.4
22'	3.94 (dd)	3.97 (dd)	—	—
NH	5.33 (s)	4.07 (s)	—	—
OCH ₃	3.68 (s)	3.86 (s)	50.8	51.2

Materials and Methods

M.p. not corrected. UV 550 S (Perkin-Elmer); IR: 298 (Perkin-Elmer); Optical rotation: 241 (Perkin-Elmer); 1H -NMR, ^{13}C -NMR: XL 300 (Varian); MS: MS 50 (Kratos).

Plant material

The roots of *Winchia calophylla* A. DC. were collected by Guo Shao Rong and Duan Hua in October 1981 from Meng Lian county, Xishuang Banna, Yunnan province, China and identified by Prof. Chen Yu Heng, Institute of Materia Medica, Chinese Academy of Sciences, Beijing, China.

Extraction and Isolation

The dried, ground roots (29.5 kg) were extracted under reflux with ethanol. The extract was concentrated and the residue dissolved in 1% hydrochloric acid. The solution was

basified with NH_4OH and successively extracted with chloroform and n -butanol. The chloroform extract was evaporated to dryness *in vacuo* to afford 15 g residue. 10 g of the residue were developed on silica gel (500 g) by dry column chromatography with CH_2Cl_2 -MeOH- Me_2CO (4 + 1 + 2) and cut into 9 equal sections. Sections 2–8 were individually eluted with ethanol and separated on preparative TLC, using CH_2Cl_2 -EtOAc-MeOH (1 + 1 + 1) to give 0.34 g of 1. The evaporated n -

butanol extract (80 g) was chromatographed on silica gel with $CHCl_3$ -MeOH with increasing MeOH content. The mixture of alkaloids 1 and 2 was obtained from fractions eluted with $CHCl_3$ -MeOH (9 + 1) and again separated on a silica gel column ($CHCl_3$ -MeOH, 9 + 1) to yield 0.18 g of 2. By elution with $CHCl_3$ -MeOH (85 + 15), 2.52 g of echitamine chloride and 10.37 g of loganine were obtained.

N_b -Demethylechitamine N -oxide (2)

a) From *W. calophylla* (see extraction and isolation).

b) To 50 mg (0.14 mmol) 1 in 1 ml EtOH, 1 ml hydrogen peroxide (30%) was added. After stirring for 24 h at room temperature, 2 ml H_2O were added and the mixture was extracted with $CHCl_3$. Evaporation of the solvent yielded 20 mg (37%) 1. White needles; m.p. 216–219 °C (MeOH); $[\alpha]_D^{20}$: -60° (c 0.46; EtOH). UV (EtOH): λ_{max} (log ϵ) 204

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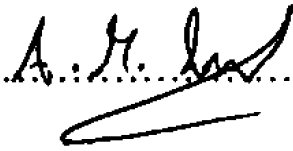
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