J. Chem. Research (S), 1998, 252–253†

Notes on Activity Tests and Constituents of two Supposed Medicinal Plants from South Africa, *Englerophytum magalismontanum* and *Diospyros lycioides desf. subsp. sericea*†

Eckehard Volker Dehmlow,*[a] Teunis Van Ree,[b] Jasmin Jakupovic,[c] Elisabeth Take[a] and Heinrich Künsting Jr[a]

[a]*Fakultät für Chemie, Universität Bielefeld, Postfach 100131, D-33501 Bielefeld, Germany*
[b]*University of Venda, Private Bag X 2220, Sibasa, South Africa*
[c]*Institut für Organische Chemie, T.U. Berlin, Str. des 17 Juni Nr 135, D-10623 Berlin, Germany*

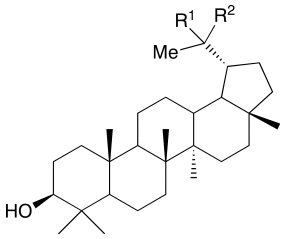
Supposed pharmacological effects of extracts from the title plants could not be substantiated, triterpenes 3β,20-lupandiol (**1a**), uvaol (**2a**) and ursolic acid (**2b**) were isolated from *Englerophytum* and 19β-lupeol (**1b**) and **2b** from *Diospyros*.

Extracts from the title plants have been used to some extent in tribal medicine by the Vhavenda in Northern Transvaal, Republic of South Africa: *Englerophytum* root extracts are supposed to help against abdominal pains and parts of the plant are used as a remedy against epilepsy. Decoctions from the other plant also find use against epilepsy and, in addition, in stopping intestinal bleeding.[1] *Englerophytum magalismontanum*, a small tree, belongs to the family sapotaceae (order ebenales) whereas *Diospyros lycioides*, 'bluebush' is a member of the ebenaceae (order ebenales). The root bark of the latter species is known to contain a number of quinoid compounds such as 7-methyljuglone, diospyrine, methylnaphthazarine and diosindigo.[2–4] For our investigation, air-dried shredded leaves, twigs and small branches were used.

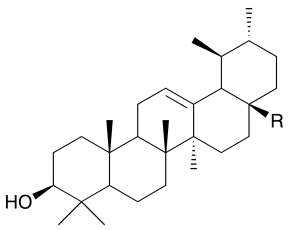
Raw extracts were prepared either by direct solvent extraction or after acid digestion followed by rough separation. These were subjected to a basic pharmacological screening in which our testing partners focused on available assays well beyond the original indications: (i) estrogenic and anti-estrogenic, gestagenic/antigestagenic activities, (ii) possible potentiation of the antiplasmodic (antimalaria) activity of chloroquine against *Plasmodium falciparum*, (iii) control of coagulation and fibrinolysis of blood, (iv) endotheline converting enzyme antagonism (control of tonus of blood vessels), (v) T3 enzyme antagonism (control of heart rhythm), (vi) serotonin agonism/antagonism (influence on psychotic processes), (vii) antagonism of glutamate receptors (regulation of glutamate level in nerve signal transfer may play a role in epilepsy, for instance), (viii) inhibition of interleukin converting enzyme (high ICE levels are found in inflammatory processes) and (ix) cytotoxicity on human carcinoma cells. Slight antigestagenic and antiplasmodic activities were found in standardized tests (i) and (ii) with extracts from *Englerophytum*, but none in the other assays. These did not warrant follow-up experiments. *Diospyros* exhibited even less significant effects in assays (i) and (ii), and none in the others. Thus, leads from tribal medicine cannot be substantiated in these cases.

Chromatographic separation of the less polar constituents of the extracts allowed the enrichment of steroid/triterpene fractions. From both plants stigmasterol and sitosterol could be isolated. These were identified by mass spectrometry (M^+, 412 and 414, respectively) and by comparison of the ^{13}C NMR spectra with known data. More importantly, fine-chromatography of the *Englerophytum* gave two main triterpene alcohols, $C_{30}H_{52}O_2$ (**A**) and $C_{30}H_{50}O_2$ (**B**) and in addition an acid, $C_{30}H_{48}O_3$ (**C**).

Structural elucidation of compound **A** was difficult because the MS spectrum exhibited $C_{30}H_{50}O$ as the apparent molecular peak, being actually owing to loss of water. As there were no indications of a sixth ring (cyclopropane?) or a double bond that formula had to be wrong. The ^{13}C NMR signals at 73.5 and 79.0 showed that two oxygen-carrying carbons must be present. Comparison with literature data allowed assignment of compound **A** as 3β,20-lupandiol (**1a**), also called monogynol A.[5] The ^{13}C NMR chemical shifts were similar to the ones of certain compounds isolated previously by one of us.[6] These carry hydroxymethyl or methoxycarbonyl groups at position 28. The assignment was corroborated by the $^1H/^{13}C$ correlations given in Table 1.



1a R^1 = OH, R^2 = Me
1b R^1, R^2 = =CH_2



2a R = CH_2OH
2b R = CO_2H

Compound **1a** is rare, having been isolated only four times before, from plants that vary widely in plant systematics: *Maclura pomifera* (urticales),[5] *Melodinus panifera* (gentianales),[7] *Betula verrucosa* (fagales)[8] and finally *Relhania calcina* (asterales).[6]

Analysis of MS and ^1H and ^{13}C NMR spectra of compound **B** showed that this must be uvaol, 28-hydroxy-α-amyrine (**2a**). In particular, a ^{13}C signal comparison with known chemical shifts of ursolic acid (**2b**) was helpful

Table 1 $^1H/^{13}C$ correlations over two and three bonds for compound **1a**

Position of H (carbon atom no.)	^2J/Hz	^3J/Hz
23	4	3, 5, 24
24	4	3, 5, 23
25	10	1, 5, 9
26	8	7, 9, 14
27	14	8, 13, 15
28	17	16, 18, 22
29	20	19, 30
30	20	19, 29

*To receive any correspondence.
†This is a **Short Paper** as defined in the Instructions for Authors, Section 5.0 [see *J. Chem. Research (S)*, 1998, Issue 1]; there is therefore no corresponding material in *J. Chem. Research (M)*.

and allowed assignment of all signals. The last isolated compound (**C**) proved to be ursolic acid itself.

A similar separation of the components from *Diospyros* gave compounds **D** ($C_{30}H_{50}O$) and **E** ($C_{30}H_{48}O_3$). Spectral analysis and comparison with known spectra indicated that compound **D** was 19β-lupeol (**1b**) and **E** was ursolic acid (**2b**).

Uvaol (**2a**) has been isolated repeatedly from various plants[9], and lupeol and ursolic acid are very common triterpenes.[9]

Experimental

Plant materials were identified by the botanist Norbert Hahn (University of Venda) in the Soutpansbergen and were collected and dried by T.v.R. in March 1992. Specimens have been deposited in the Herbarium Soutpansbergensis (Sibasa).

Raw extracts for Pharmological Testing.—Plant material was extracted either with EtOH or with light petroleum. The concentrated solutions were chromatographed coarsely on silica gel consecutively with mixtures of increasing solvent polarity. Alternatively the samples were treated with cold 2% aqueous HOAc or with ethanol/acetic acid (9:1) at 80 °C or 2 M HCl at 100 °C, filtered, then neutralized with aqueous NH_3 and filtered again. Both filtrate and precipitate were extracted with ethyl acetate, then coarsely separated as above.

Isolation of Triterpenes from Englerophytum.—The plant material (1 kg) was Soxhlet-extracted with light petroleum (bp 45–60 °C) for 20 h, then with CH_2Cl_2. Some 12.3 g of the light petroleum extract was then chromatographed repeatedly on silica gel with diethyl ether–light petroleum (7:3) using Komarowsky's reagent for the visualization of zones. Ultimately, separation resulted in the isolation of 200 mg of **1a**, about 200 mg of **2a** in pure form and 40 mg of stigmasterol (containing some sitosterol). Repeated chromatography of the CH_2Cl_2 extract with diethyl ether–light petroleum (6:4) gave a fraction rich in **2b**. Acetylation permitted the separation of pure acetyl-**2b** (26 mg).

The main Triterpenes from Diospyros.—These were isolated in a similar manner: **1b** (pure: 48 mg from 1 kg) and a sitosterol/stigmasterol mixture (24 mg from 1 kg) from the light petroleum extract, and **2b** (60 mg from 1 kg) from the EtOH extract by repeated fine-chromatography.

3β,20-*Lupandiol* **1a**.—Mp 213 °C (lit. 233–238 °C[5]); δ_H (250 MHz; $CDCl_3$) 0.76 (s, 3 H), 0.81 (s, 3 H), 0.84 (s, 3 H), 0.95 (s, 3 H), 0.97 (s, 3 H), 1.06 (s, 3 H), 1.12 (s, 3 H), 1.22 (s, 3 H), 1.25–1.90 (m, 24 H), 3.19 (m, 1 H); δ_C (Bruker AC 250 P; 62.896 MHz; $CDCl_3$) 14.9 (C-27), 15.4 (C-24), 16.2 (C-25 + C-26), 18.4 (C-6), 19.2 (C-28), 21.4 (C-11), 24.8 (C-29), 27.4 (C-2), 27.6 (C-15), 28.0 (C-23), 28.7 (C-21), 29.1 (C-12), 31.6 (C-30), 34.6 (C-7), 35.6 (C-22), 37.1 (C-10), 37.5 (C-13), 38.8 (C-1), 38.9 (C-4), 40.2 (C-16), 41.4 (C-8), 43.6 (C-14), 44.7 (C-17), 48.4 (C-18), 50.0 (C-19), 50.3 (C-9), 55.3 (C-5), 73.5 (C-20), 79.0 (C-3); *m/z* 426 (M – H_2O), 411, 218, 207, 189, 135, 95 (base peak). (Found: C 81.34; H, 11.79. $C_{30}H_{52}O_2$ requires C, 81.05; H, 12.17%)

Lupeol **1b**.—δ_C ($CDCl_3$) 14.6 (C-27), 15.4 (C-24), 16.0 (C-25), 16.1 (C-26), 18.0 (C-28), 18.4 (C-6), 19.3 (C-30), 21.0 (C-11), 23.5 (C-2), 25.2 (C-12), 27.5 (C-15), 28.0 (C-23), 29.9 (C-21), 34.4 (C-10), 35.6 (C-16), 37.2 (C-10), 38.1 (C-13), 38.8 (C-1), 38.9 (C-4), 40.0 (C-22), 40.9 (C-8), 42.9 (C-14), 43.0 (C-17), 48.0 (C-19), 48.4 (C-18), 50.5 (C-9), 55.4 (C-5), 79.0 (C-3), 109.3 (C-29), 150.9 (C-20) (cf. lit.[10]).

Uvaol **2a**.—Mp 208 °C (lit. 222–224 °C[11]); δ_H($CDCl_3$) 0.97 (s, 3 H), 0.95 (s, 3 H), 0.93 (s, 3 H), 0.82 (d, 3 H), 1.00 (s, 6 H), 1.10 (s, 3 H), 1.15–2.00 (m, 21 H) 3.19 (m, 2 H), 3.53 (m, 1 H), 5.14 (t, 1 H, J = 3.5 Hz); δ_C ($CDCl_3$) 15.6 (C-24), 15.7 (C-25), 16.80 (C-26), 17.4 (C-29), 18.4 (C-6), 21.3 (C-30), 23.34 (C-27), 23.36 (C-11), 23.41 (C-16), 26.05 (C-2), 27.3 (C-15), 28.2 (C-23), 30.7 (C-21), 32.8 (C-7), 35.2 (C-22), 36.9 (C-10), 38.0 (C-4), 38.80 (C-8), 38.84 (C-1), 39.4 (C-20), 39.45 (C-19), 40.1 (C-14), 42.1 (C-17), 47.7 (C-9), 54.1 (C-18), 55.2 (C-5), 69.9 (C-28), 79.0 (C-3), 125.1 (C-12), 38.7 (C-13); *m/z* (CI) 441 (M – 1), 203 (base peak); *m/z* (EI) 411 (M – CH_2OH), 234, 203 (base peak) 189, 133.

Diacetyluvaol.—Mp 147–148 °C (lit. 148–150 °C[11]).

Ursolic acid **2b**.—mp 272 °C (lit. 281–286 °C[12]). Although the ^{13}C NMR spectrum showed the signals known from the literature,[13,14] the compound was impure. Acetylation yielded the 3-β-acetyl derivative, mp 272 °C (lit. 268 °C,[15] 286–293 °C[13,14]).

This work was supported by the Deutsche Forschungsgemeinschaft and Fonds der Chemischen Industrie. For the pharmacological tests [assay no.], special thanks are due to Dr. Henry Laurent (Schering A. G., Berlin [(i)]), Dr. Francois (Institute de Médicine Tropicale Prince Léopold, Antwerp, [ii]) and Drs Schirmer and Witschel (BASF, Ludwigshafen, [iii–ix]).

Received, 17th November 1997; Accepted, 22nd December 1997
Paper E/7/08239H

References

1 D. E. N. Mabogo, M.Sc Thesis, University of Pretoria, 1990.
2 M. A. Ferreira, M. A. C. Costa and A. C. Alves, *Phytochemistry*, 1972, **11**, 2352.
3 A. C., Alves, M. A. C. Costa and M. A. Ferreira, *Ann. Fac. Farm. Porto*, 1973, **33**, 5 (*Chem. Abstr*., 1976, **85**, 17 097).
4 M. A. Ferreira, M. A. C. Costa and A. C. Alves, *Plant Med. Phytother*., 1972, **6**, 32 (*Chem. Abstr*., 1972, **77**, 58 832).
5 K. G. Lewis, *J. Chem. Soc*., 1959, 73.
6 F. Tsichritsis and J. Jakubovic, *Phytochemistry*, 1990, **29**, 3173. The authors should like to correct the inadvertently interchanged assignments of C-16 and C-22 of compound **56a** in the original paper (Table 14, p. 3183).
7 S. K. Chatterjee, N. Anand and M. L. Dhar, *J. Sci. Ind. Res.* (*India*), 1959, **18b**, 262.
8 B. O. Lindgreen and C. M. Svahn, *Acta Chem. Scand*., 1966, **20**, 1720.
9 J. S. Glasby, in *Dictionary of Plants Containing Secondary Metabolites*, Taylor & Francis, London, 1991.
10 R. A. Paupetit, J. M. Waters, D. D. Rowan, G. B. Russell, H. E. Connor and A. W. Purdie, *Aust. J. Chem*., 1984, **37**, 1341.
11 A. Zürcher, O. Jeger and L. Ruzicka, *Helv. Chim. Acta*, 1954, **37**, 2145.
12 G. H. Stout and K. L. Stevens, *J. Org. Chem*., 1963, **28**, 1259.
13 S. Soe, Y. Tomita and K. Tori, *J. Am. Chem. Soc*., 1981, **103**, 2075.
14 P. J. Houghton and L. Lian, *Phytochemistry*, 1986, **25**, 1939.
15 R. Dayal, *J. Indian Chem. Soc*., 1982, **59**, 1008.