2. Johnson, E. S., Kadam, N. P., Hylands, D. M. and Hylands, P. J. (1985) *Brit. Med. J.* **291**, 569.
3. Berry, M. I. (1984) *Pharm. J.* **232**, 611.
4. Editorial (1985) *The Lancet* 1084.
5. Groenewegen, W. A., Knight, D. W. and Heptinstall, S. (1986) *J. Pharm. Pharmacol.* **38**, 709.
6. Kupchan, S. M., Fessler, D. C., Eakin, M. A. and Giacobbe, T. J. (1970) *Science* **168**, 376.
7. Bohlmann, F. and Zdero, C. (1982) *Phytochemistry* **21**, 2543.
8. Fischer, N. H., Olivier, E. J. and Fischer, H. D. (1979) *Prog. Chem. Org. Nat. Prod.* **38**, 47.
9. Kelsey, R. G., Shafizadeh, F., Campbell, J. A., Craig, A. C., Campana, C. F. and Craig, R. E. R. (1983) *J. Org. Chem.* **48**, 125.
10. Lee, K. H., Simpson, R. F. and Geissman, T. A. (1969) *Phytochemistry* **8**, 1515.
11. Balci, M. (1981) *Chem. Rev.* 91.
12. Osawa, T., Taylor, D., Suzuki, A. and Tamura, S. (1977) *Tetrahedron Letters* 1169.

Phytochemistry, Vol. 28, No. 3, pp. 943–945, 1989.
Printed in Great Britain.

0031-9422/89 $3.00+0.00

A TRITERPENE ALCOHOL, LANSIOL, FROM *CLAUSENA LANSIUM*

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(*Received* 24 *June* 1988)

Key Word Index—*Clausena lansium*; Rutaceae; aerial portion; triterpene; 3β-hydroxy-23,24,24-trimethyllanosta-9(11)-25-diene; structural analysis.

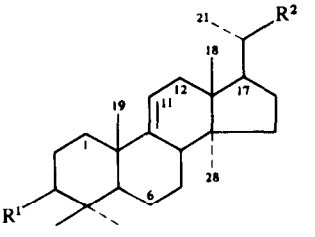
Abstract—A new tetracyclic triterpene alcohol characterized as 3β-hydroxy-23,24,24-trimethyllanosta-9(11)-25-diene has been isolated from the aerial parts of *Clausena lansium*.

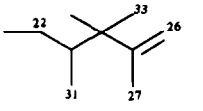
INTRODUCTION

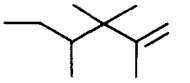
Our continued interest in the chemistry of new constituents from *Clausena* species [1–7] prompted us to study the chemical constituents of *Clausena lansium* (Lour.) Skeels (Syn *C.wampi* Olive) (Rutaceae). The ethanolic extract of the leaves of this plant on fractionation by a combination of column chromatography and PLC on silica gel of the hexane fraction afforded a new tetracyclic triterpene alcohol designated as lansiol. The structural analysis of lansiol is the subject of the present communication.

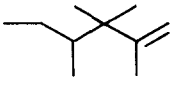
RESULTS AND DISCUSSION

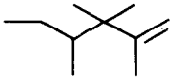
Lansiol (1), mp. 197–198° ($CHCl_3$–MeOH) $[\alpha]_D^{25}+83°$ ($CHCl_3$; *c* 1), ν_{max}^{KBr} 3350 cm^{-1} (OH) confirmed by the formation of the mono acetate and Collin's oxidation to give a ketone (lansione-3). The presence of a vinylidene group was indicated at 1648 and 890 cm^{-1}. It showed a molecular ion peak at *m/z* 468 in its mass spectrum corresponding to the molecular formula $C_{33}H_{56}O$. This, coupled with the presence of nine methyl signals between δ 0.68 and 1.03 and one vinylic methyl singlet at δ 1.55, confirmed its terpenoid nature. There was one proton

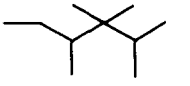


1 R^1 = R^2 = 

2 R^1 = R^2 = 

3 R^1 = O R^2 = 

4 R^1 = R^2 = 

5 R^1 = R^2 = 

Part 2 in the series 'Chemical Constituents of *Clausena lansium*'.

*CDRI Communication No. 4308.

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Table 1. ^{1}H NMR chemical shifts (δ values, $CDCl_3$, TMS standard, 400 MHz) of lansiol and *O*-methylclausenol

H	Lansiol (**1**)	*O*-Methylclausenol (**4**)
18	0.68 (*s*, 3H)	0.59 (*s*, 3H)
19	1.00 (*s*, 3H)	0.96 (*s*, 3H)
21	1.03 (*d*, J = 6.4 Hz, 3H)	0.93 (*d*, J = 6.4 Hz, 3H)
27	1.55 (*s*, 3H)	1.60 (*s*, 3H)
28	0.84 (*s*, 3H)	0.85 (*s*, 3H)
29	1.03 (*s*, 3H)	1.03 (*s*, 3H)
30	0.88 (*s*, 3H)	0.86 (*s*, 3H)
31	0.70 (*d*, J = 6.4 Hz, 3H)	0.70 (*d*, J = 6.4 Hz, 3H)
32	0.76 (*s*, 3H)	0.78 (*s*, 3H)
33	0.74 (*s*, 3H)	0.70 (*s*, 3H)
3	3.68 (*m*, 1H)	2.46 to 2.78 (*m*, 1H)
11	5.22 (*m*, 1H)	5.22 (*m*, 1H)
26	4.78 (*d*, J = 1.5 Hz, 2H)	4.70 (*d*, J = 1.5 Hz, 2H)

multiplet centred at δ 3.68, the shape and position of which corresponded very closely to the axial C_3–H in cycloartenol, 24-methylene-cycloartanol [8] and C_{32} terpenoids isolated from *Neolistea pulchella* [9]. This implied that the hydroxyl group attached to C-3 is equatorial. Lansiol formed a monoacetate, confirming that only one hydroxyl group is present in it. The chemical shift at δ 5.22 attributed to the proton $\Delta^{9(11)}$ was substantiated by the appearance of fragment ions [8–11] at *m/z* 287, 273 and 261 in its mass spectrum as well as by its sluggish behaviour towards catalytic hydrogenation under normal conditions. Another relevant feature of the mass spectrum of (**1**) was the presence of a conspicuous $[M]^+$ at *m/z* 468 followed by the base peak at *m/z* 313 which accounted for the M-side chain -2H fragment [7–10]. Further structural confirmation was obtained by Collins oxidation product (**3**), methylation product of lansiol (**1**) and demethylation of *O*-methylclausenol (**4**). The methoxy compound obtained from the methylation of lansiol was found to be completely identical (Co-TLC, mp, mmp, superimposable IR and ^{13}C NMR data) to *O*-methylclausenol. The hydroxy compound obtained by demethylation of *O*-methylclausenol was also found to be identical to lansiol (co-TLC, mp, mmp and superimposable IR spectra). Further support for the structure was obtained by comparison of ^{1}H NMR and ^{13}C NMR of lansiol, lansione and *O*-methylclausenol (Tables 1 and 2).

Table 2. ^{13}C NMR chemical shift values of lansiol, lansione and *O*-methylclausenol (δ, values $CDCl_3$, TMS standard, 100 MHz)

C	Lansiol (**1**)	Lansione (**3**)	*O*-Methylclausenol (**4**)
1	36.22 *t*	36.60 *t*	36.33 *t*
2	27.90 *t*	34.86 *t*	27.97 *t*
3	78.95 *d*	217.05 *s*	88.70 *d*
4	39.17 *s*	47.69 *s*	39.12 *s*
5	50.89 *d*	50.98 *d*	53.17 *d*
6	18.56 *t*	18.53 *t*	19.3 *t*
7	28.26 *t*	27.74 *t*	28.19 *t*
8	41.89 *d*	53.46 *d*	36.70 *d*
9	158.00 *s*	157.67 *s*	150.18 *s*
10	39.45 *s*	37.60 *s*	39.49 *s*
11	115.03 *d*	116.36 *d*	114.88 *d*
12	37.21 *t*	37.19 *t*	36.15 *t*
13	44.33 *s*	44.25 *s*	44.37 *s*
14	47.07 *s*	46.98 *s*	47.07 *s*
15	30.91 *t*	36.74 *t*	30.37 *t*
16	27.52 *t*	33.92 *t*	29.68 *t*
17	52.60 *d*	50.78 *d*	51.03 *d*
18	14.42 *q*	14.41 *q*	14.42 *q*
19	22.32 *q*	22.03 *q*	22.27 *q*
20	36.67 *d*	36.74 *d*	37.28 *d*
21	18.56 *q*	18.44 *q*	18.53 *q*
22	37.21 *t*	36.60 *t*	32.22 *t*
23	37.66 *d*	37.36 *d*	41.93 *d*
24	33.99 *s*	33.92 *s*	33.91 *s*
25	148.57 *s*	147.08 *s*	148.76 *s*
26	106.45 *t*	106.08 *t*	111.088 *t*
27	13.09 *q*	13.06 *q*	16.40 *q*
28	25.74 *q*	27.98 *q*	22.27 *q*
29	27.98 *q*	27.95 *q*	28.06 *q*
30	15.68 *q*	14.41 *q*	14.42 *q*
31	21.45 *q*	21.78 *q*	21.33 *q*
32	27.02 *q*	27.48 *q*	22.27 *q*
33	23.40 *q*	23.39 *q*	22.64 *q*
34 (OMe)	—	—	57.42 *q*

EXPERIMENTAL

Mps: uncorr. ^{1}H NMR 400 MHz, $CDCl_3$, TMS as int. standard; ^{13}C NMR, 100 MHz.

Extraction and isolation. Plant material was collected from H.R.I., Saharanpur in April 1976. The air-dried powdered leaves (2.5 kg) were extracted with 95% EtOH and concd, the EtOH extract was fractionated into hexane, C_6H_6, EtOAc, *n*-BuOH and aq. fractions. On chromatography of the hexane fraction (15 g) over a column of neutral alumina, the 25% C_6H_6–hexane eluents afforded lansiol (**1**) which was further purified on prep. silica gel plates impregnated with 10% $AgNO_3$ soln.

Lansiol (**1**). Colourless crystals (mp, 197–198°), UV λ_{max}^{MeOH} 226 nm; IR ν_{max}^{KBr} cm^{-1} 3350, 2900, 2850, 1648, 1460, 1365, 1275, 1095, 1040, 980, 890, 775, 720 and 670 cm^{-1}; ^{1}H NMR (see Table 1). MS: High resolution, *m/z* found 468.4326 (42.6%) which corresponds to $C_{33}H_{56}O$.

Lansiol acetate (**2**). Lansiol (10 mg) was kept at room temp. overnight with acetic anhydride and pyridine, On work-up, it gave lansiol monoacetate crystals, mp 190–192°, molecular formula $C_{35}H_{58}O_2$; M^+ *m/z* 510; IR ν_{max}^{KBr} 1735 cm^{-1} (for >C=O of the acetate gp).

Lansione (**3**). Lansiol (100 mg) was dissolved in CH_2Cl_2 (10 ml) and 150 mg of the Collins reagent was added to the reaction mixture, it was stirred for 4 hr, purified by column chromatography over silica gel, and crystallized with $CHCl_3$ (80 mg), mp 144–146°; M^+, *m/z* 466; IR ν_{max}^{KBr} 2970, 2930, 2870, 1710, 1695, 1460, 1375, 1110 and 890 cm^{-1}; ^{13}C NMR (see Table 2).

Methylation of lansiol. Lansiol (6 mg) taken in DMSO (5 ml) was treated with NaH (0.5 g). Stirring was continued at room temp. for 4 hr, and 1.5 ml of MeI was added to the cooled reaction mixture before stirring. Excess of NaH was decomposed with EtOH and the reaction mixture was poured into ice-cold H_2O and the product was extracted with $CHCl_3$ which was purified on silica gel plates (2 mg), mp 182–183°, molecular formula $C_{34}H_{58}O$; M^+, *m/z* 482, methoxy proton signal at δ 3.36 in ^{1}H NMR of the methylated product, superimposable IR with that of *O*-methylclausenol (**4**).

Demethylation of O-methylclausenol. O-Methylclausenol (**4**) (10 mg) and freshly prepared pyridine hydrochloride (0.5 g) was refluxed for 4 hr at 180–200° in an oil bath. The reaction mixture

was worked-up as usual and the product was further purified by PLC over silica gel plates using C_6H_6–hexane as eluents. The purified demethylated product (yield 6 mg) was found to be completely identical to lansiol (**1**) isolated from *C. lansium*, on TLC, mp, mmp and superimposable IR spectrum.

Reduction of lansiol. A mixture of (**1**) (50 mg) dissolved in $CHCl_3$ (20 ml) and Adam's catalyst (25 mg) was stirred for 2 hr at room temp. in an atmosphere of H_2. The solvent was removed and the product (**5**) was crystallized with MeOH–$CHCl_3$; yield, 48 mg; mp 198–200°; M^+ *m/z* 470; $[\alpha]_D^{25}$ +51.5 ($CHCl_3$; *c* 0.8).

Acknowledgement—We are grateful to Dr D. Prakash for his association in early stages of this work.

REFERENCES

1. Manandhar, M. D., Shoeb, A., Kapil, R. S. and Popli, S. P. (1977) *Experientia* **33**, 153.
2. Prakash, D., Raj, K., Kapil, R. S. and Popli, S. P. (1978) *Phytochemistry* **17**, 1194.
3. Prakash, D., Raj, K., Kapil, R. S. and Popli, S. P. (1980) *Indian J. Chem.* **19**, 1075.
4. Lakshmi, V., Raj, K., Kapil, R. S. and Patnaik, G. K. (1987) *Indian Drugs* **24**, 285.
5. Lakshmi, V., Prakash, D., Raj, K. and Kapil, R. S. (1984) *Phytochemistry* **23**, 2629.
6. Lakshmi, V., Raj, K., Kapil, R. S. and Patnaik, G. K. (1988) *Indian Drugs* (in press).
7. Prakash, D., Lakshmi, V., Raj, K. and Kapil, R. S. (1989) *Fitoterapia* (in press).
8. Ritchie, E., Senior, R. G. and Taylor, W. C. (1969) *Aust. J. Chem.* **22**, 2371.
9. Hui, W. H., Luke, K., Arthur, H. R. and Loo, S. N. (1971) *J. Chem. Soc.* (*C*) 2826.
10. Vorbruggen, H., Pakrashi, S. C. and Djerassi, C. (1963) *Annalen* **57**, 668.
11. Wyllie, S. G. and Djerassi, C. (1968) *J. Org Chem.* **33**, 305.

Phytochemistry, Vol. 28, No. 3, pp. 945–947, 1989.
Printed in Great Britain.

0031-9422/89 $3.00+0.00

ERGOSTA-7,22-DIEN-3β-OL GLYCOSIDE FROM *TYLOPILUS NEOFELLEUS*

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(*Received in revised form* 27 *July* 1988)

Key Word Index—*Tylopilus neofelleus*; Basidiomycete; higher fungi; glycoside; ergosta-7,22-dien-3β-*O*-glucopyranoside.

Abstract—A new glycoside of ergosta-7,22-dien-3β-ol was isolated from the fresh fruit body of *Tylopilus neofelleus* and its structure was established by chemical and spectroscopic means. Basidiomycetes produce many ergosterol analogues, but the presence of the glycoside of an ergosterol derivative is the first finding from a natural source.

INTRODUCTION

We have investigated the constituents of Basidiomycetes and elucidated the structure of three new lanostane triterpenes having a δ-lactone in the side chain from *Astraeus hygrometricus* [1], and some ergosterol analogues from *Inonotus mikadoi* [2]. Now, we have carried out an investigation of the constituents of *Tylopilus neofelleus* (Boletaceae). In this paper we wish to report the structural elucidation of a new glycoside, ergosta-7,22-dien-3β-*O*-glucopyranoside (**2**).

RESULTS AND DISCUSSION

The methanol extract from the fresh fruit bodies of *T. neofelleus* Hongo was partitioned with water and ethyl acetate. The ethyl acetate extract was separated using silica gel column chromatography to give compounds **1**–**3**.

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Compound **1**, $C_{28}H_{46}O$, showed the presence of two tertiary methyls, four secondary methyls, one proton attached to an oxygen bearing carbon and three protons attached to double bond in the ^1H NMR spectrum. Its decoupled ^{13}C NMR spectrum contained 28 peaks (Table 1). In the mass spectrum of **1**, fragment ion peaks were very similar to those of an ergosterol derivative [3]. These facts indicated that compound **1** was ergosta-7,22-dien-3β-ol, confirmed by direct comparison with literature data [4].

Compound **2**, $C_{34}H_{56}O_6$, showed absorption at 3500 (OH) cm^{-1} in the IR spectrum. The ^1H NMR spectrum of **2** showed the presence of two tertiary methyls, four secondary methyls, and a glycoside moiety. The decoupled ^{13}C NMR spectrum (Table 1) of **2** contained 34 signals, six of which were assigned to a glucose moiety [5]. The remaining signals were very similar to those of compound **1** except the signals at δ 30.1 (*t*), 77.5 (*d*), and 34.9 (*t*). This suggested that the structure of compound **2** was ergosta-7,22-dien-3β-*O*-glucoside, and the ^{13}C NMR differences (a downfield shift of the signal at δ