Phytochemistry, Vol. 23, No. 8, pp. 1699–1702, 1984.
Printed in Great Britain.

0031–9422/84 $3.00 + 0.00

TWO NOR-TRITERPENE LACTONES FROM *CALTHA PALUSTRIS**

PRABHA BHANDARI and R. P. RASTOGI

Central Drug Research Institute, Lucknow-226 001, India

(*Revised received* 11 *November* 1983)

Key Word Index—*Caltha palustris*; Ranunculaceae; triterpene lactones; caltholide; 24-nor-3β-hydroxylupan-13β,28-lactone; epicaltholide; 24-nor-3α-hydroxylupan-13β,28-lactone.

Abstract—The chemical constituents present in the chloroform-soluble fraction of the ethanolic extract of *Caltha palustris* have been investigated. Two of these, caltholide and epicaltholide have been characterized as 24-nor-3β-hydroxylupan-13β,28-lactone and its 3α-isomer, respectively.

INTRODUCTION

Caltha palustris L. (Ranunculaceae) is a herb, commonly distributed throughout the Western Himalayas up to an altitude of about 3000 m. The roots are reported to be acrid and poisonous and contain helleborin and veratrin [1]. The plant is reported to yield scopoletin, umbelliferone [2] and triterpene glycosides [3]. The total glycoside mixture has been tested for various biological activities in rats [4–6].

RESULTS AND DISCUSSION

An aqueous methanolic solution of the plant extract was successively fractionated into chloroform and butanol soluble fractions. The chloroform fraction showed seventeen spots including some minor components on TLC which were designated alphabetically as substances A to O in decreasing order of R_f values. These constituents were separated by chromatography over silica gel and by preparative TLC. Two of the substances, C and D, are novel triterpene lactones, now named as epicaltholide and caltholide, and the present paper deals with the characterisation of these two compounds.

Caltholide (compound D, **1a**), mp $> 310°$ dec., $C_{29}H_{46}O_3$ (M^+ at m/z 442.3506), gave a positive Liebermann Burchard test for triterpenes. The IR spectrum showed absorptions for a γ-lactone (1755 cm^{-1}) and a hydroxyl group (3350 cm^{-1}). Its ^1H NMR spectrum exhibited signals for six methyl groups in the region δ0.78–1.11 and a carbinolic proton at δ3.7. The absence of any additional signal near δ4.0 indicated that the γ-lactone terminated at a tertiary centre.

Caltholide gave a monoacetate **1c** whose ^1H NMR spectrum exhibited an acetoxymethyl signal at δ2.03 and the carbinolic proton at δ4.79 which confirmed the presence of a secondary hydroxyl group in the molecule. The mass spectral fragmentation pattern of caltholide and its acetyl derivative indicated that it belonged to the lupane series [7]. The fragment ions at m/z 206.1686 ($C_{14}H_{22}O$) and 193.1512 ($C_{13}H_{21}O$) from **1a** and corresponding ions at m/z 248 and 235 from **1c**, and the common ions 188.1537 ($C_{14}H_{20}$) and 175.1504 ($C_{13}H_{19}$) from both, comprise of rings A/B which confirmed the presence of a lactone group in rings C/D. The base peak at m/z 206 also restricted the location of the hydroxyl group at C-3 and the nor-position in rings A/B (Scheme 1).

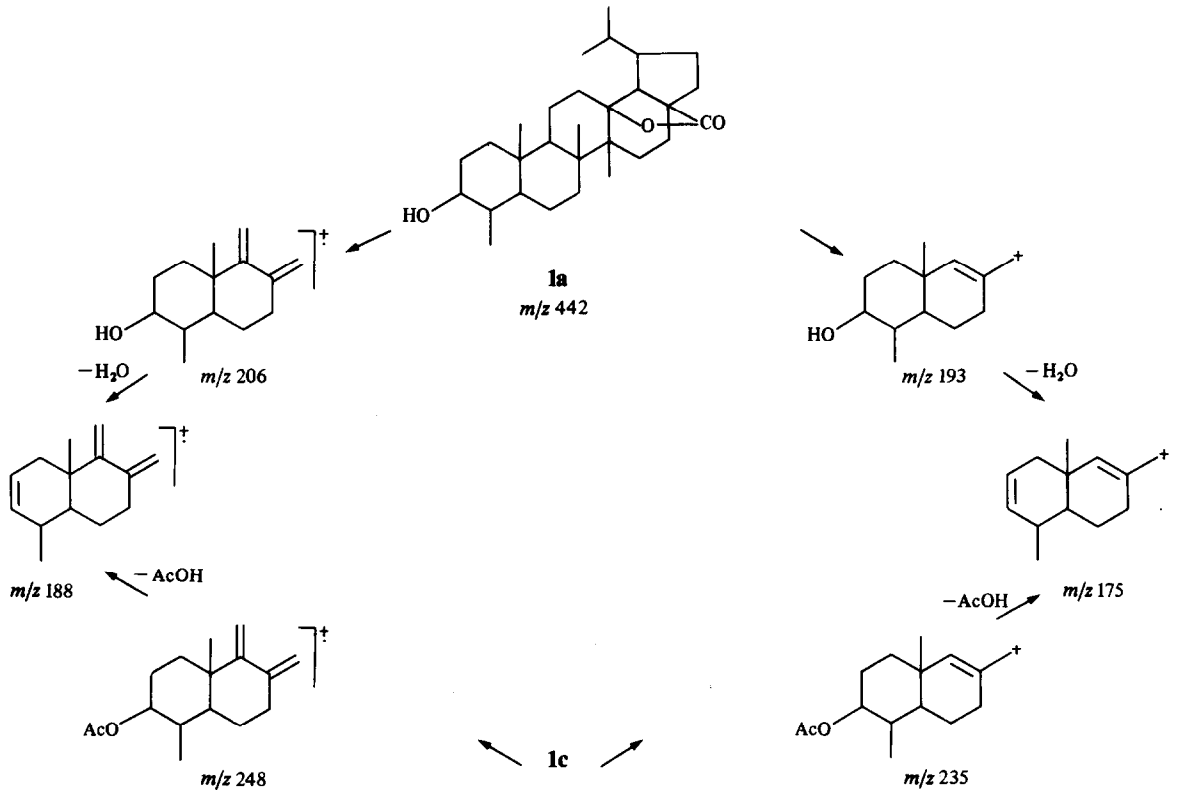
The pyridinium–chlorochromate oxidation [8] of caltholide yielded a keto derivative **1d** which showed carbonyl absorption at 1700 cm^{-1} (six-membered ring ketone) in its IR spectrum, thus supporting C-23 or C-24 as the nor-position in the molecule.

For assignments in the methyl region [9] of the ^1H NMR (400 MHz) spectrum of caltholide acetate, the most downfield signal at δ1.16 was due to the C-26 methyl group which was attributed to the diamagnetic anisotropic effect of the lactone group while C-27 methyl group appeared at 1.13. The two methyls of the isopropyl group resonated as a singlet at 0.87. The appearance of a doublet (J = 7 Hz) at 0.85 due to a secondary methyl further confirmed C-24 as the nor-position thus excluding the possibility of nor-methyl at the C-8 or C-10 positions. The C-25 methyl group appeared at δ0.82. The carbinolic proton geminal to the acetoxy group appeared as a doublet of triplets at 4.79 due to coupling with methine at C-4 and methylene protons at the C-2 position which was compatible with its α (axial) disposition. Thus, the hydroxyl must have the β (equatorial) configuration. The multiplicity ($J_{3ax,4ax}$ = 12 Hz) also confirmed the existence of an equatorial methyl group at the C-4 position.

Caltholide was reduced with lithium aluminium hydride to give a triol derivative **2a** whose IR spectrum was devoid of carbonyl absorption. The acetylation of the triol afforded a diacetyl derivative **2b** whose IR spectrum (3480 cm^{-1}) indicated the presence of a tertiary hydroxyl group. The ^1H NMR spectrum exhibited a singlet for two acetoxymethyls at δ1.96 and the carbinolic protons as an AB quartet at 4.08 and 4.65 (each d, J = 12 Hz) due to the methylol group and at 4.72 (1H) corresponding to a secondary hydroxyl group.

The diacetate **2b** was smoothly dehydrated with BF_3-etherate to a product **3** which gave a yellow colour with tetranitromethane and showed IR absorption for a trisubstituted double bond. Its ^1H NMR spectrum exhibited a signal for an olefinic proton at δ5.1 and the mass spectrum showed M^+ at m/z 512. The base peak at m/z 203 arising from retro-Diels–Alder fragmentation further confirmed

*CDRI Communication No. 3366.



the presence of a tertiary hydroxyl group generated by the reductive cleavage of the lactone ring at C-13. The dehydrated product **3** on SeO_2 oxidation yielded a heteroannular diene **4** whose UV showed maxima at 243, 252 and 262 nm. Thus, the Δ^{12} position of the double bond in the product **3** was established. These results confirmed the structure of caltholide as 24-nor-3β-hydroxylupan-13β,28-lactone (**1a**).

Epicaltholide (Compound C, **1b**), mp 243–246°, analysed for $C_{29}H_{46}O_3$ (M^+ at *m/z* 442). The IR spectrum exhibited absorptions for hydroxyl (3250 cm^{-1}) and γ-lactone (1742 cm^{-1}) and the ^1H NMR spectrum showed signals for six methyls in the region δ0.78–1.20 and a carbinolic proton at 3.72 as a broad singlet. It afforded an acetyl derivative whose ^1H NMR spectrum exhibited an acetoxymethyl signal at 2.02 and the carbinolic proton appeared at 4.72 with $W_{1/2}$ = 7 Hz which was compatible with its equatorial configuration. The hydroxyl must, therefore, be α (axial).

The mass spectra of epicaltholide and its acetyl derivative were found to be identical with those of caltholide and its acetyl derivative. Thus, it appeared that epicaltholide was an isomer of caltholide differing in the stereochemistry of the hydroxyl group at C-3.

The pyridinium chlorochromate oxidation of epicaltholide yielded a keto derivative (M^+ at *m/z* 440), mp 228°, which was found to be identical with the keto derivative obtained similarly from caltholide. Epicaltholide was, therefore, characterized as 24-nor-3α-hydroxylupan-13β,28-lactone (**1b**).

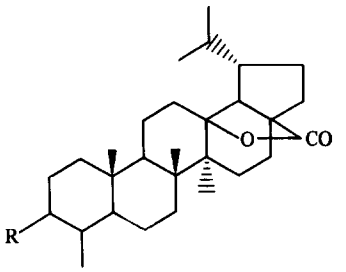
EXPERIMENTAL

The reported mps are uncorr. The ^1H NMR spectra were recorded in $CDCl_3$ unless stated otherwise, with TMS as internal standard. The plant material was collected from Garhwal region of U.P., India, and was identified by Dr. B. N. Mehrotra. A voucher specimen of the plant (No. 3308) is kept in the herbarium of the Institute.

Isolation procedure. The EtOH extract of the dried powdered plant material (5 kg) was partitioned between $CHCl_3$ and 50% aq. MeOH to give a $CHCl_3$-soluble fraction (120 g) which showed a complex mixture of 17 spots on TLC which were designated as substances A to O in decreasing order of their R_f values. This fraction was subjected to column chromatography over silica gel (1.2 kg) using hexane with progressively increasing proportions of EtOAc. The eluates (500 ml each) were collected and combined on the basis of TLC examination. The dark green residue from fractions 42–56 (6.6 g) was decolourized by passing through a charcoal column in MeOH soln. The eluate was concd to a residue which was subjected to preparative TLC ($CHCl_3$–MeOH, 98:2) to give substance C (0.08 g), substance D (0.13 g) and substance E (0.08 g) mp 202°.

Substance D (*caltholide*, **1a**). Colourless needles, mp > 310° dec. IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 2900, 1755 (γ-lactone), 1450, 1440, 1385, 1360, 1220, 1180, 1110, 1040, 980, 910. ^1H NMR: δ0.78 (3H, *s*, Me), 0.81 (6H, *s*, 2Me), 0.86 (3H, *s*, Me), 1.08, 1.11 (3H each, *s*, 2Me), 3.7 (1H, *m*, –CHOH). MS *m/z*: 442.3506 $[M]^+$ ($C_{29}H_{46}O_3$), 424.3378 ($C_{29}H_{44}O_2$), 409.3138 ($C_{28}H_{41}O_2$), 235.1673 ($C_{15}H_{23}O_2$), 206.1686 (base peak, $C_{14}H_{22}O$), 193.1512 ($C_{13}H_{21}O$), 189.1565 ($C_{14}H_{21}$), 188.1537 ($C_{14}H_{20}$), 175.1504 ($C_{13}H_{19}$).

Caltholide acetate (**1c**). Caltholide (30 mg) was reacted overnight in pyridine and Ac_2O (0.5 ml each) at room temp. After usual work up, the residue was crystallized from MeOH (28 mg), mp > 300° dec. IR ν_{max}^{KBr} cm^{-1}: 1755 (γ-lactone), 1735 (OAc). ^1H NMR (400 MHz): δ0.82 (3H, *s*, Me), 0.85 (3H, *d*, *J* = 7 Hz, Me), 0.87 (6H, *s*, 2Me), 1.13, 1.16 (3H each, *s*, 2Me), 2.04 (3H, *s*,

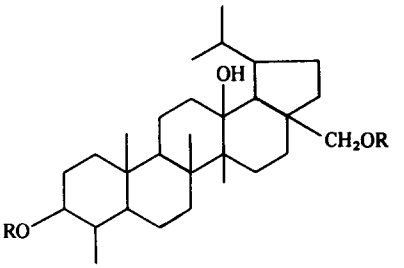


1a R = 

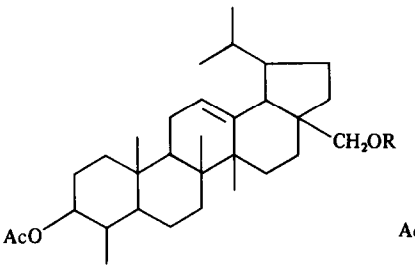
1b R = 

1c R = 

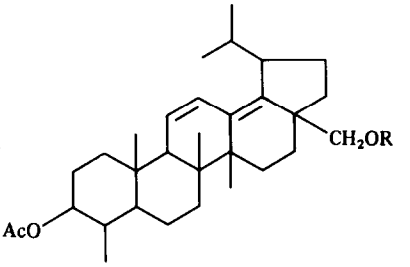
1d R = O



2a R = R¹ = H
2b R = R¹ = Ac



3



4

OCOMe), 4.79 (1H, *dt*, $J_{3ax,4ax}$ = 12 Hz, $J_{3ax,2ax}$ = 10 Hz, $J_{3ax,2eq}$ = 5 Hz, CHOAc). MS *m/z*: 484 $[M]^+$, 469, 424 $[M - AcOH]^+$, 409 $[M - AcOH - Me]^+$, 248 (base peak), 235, 189, 188, 175, 173, 161, 147, 133, 121, 119, 107.

Oxidation of caltholide. A soln of caltholide (30 mg) in CH_2Cl_2 containing pyridinium chlorochromate (30 mg) was stirred for 2 hr at room temp. It was filtered through silica gel and the residue (28 mg) crystallized from MeOH, mp 228°. IR ν_{max}^{KBr} cm^{-1}: 2955, 2922, 2850, 1750 (γ-lactone), 1700 (CO), 1460, 1440, 1380, 1255, 1220, 1085, 800. MS *m/z*: 440 $[M]^+$, 235, 204 (base peak), 191, 189, 169, 155, 141, 127 and 111.

Lithium aluminium hydride reduction of caltholide. Caltholide (30 mg) was refluxed in dry dioxane (10 ml) containing $LiAlH_4$ (50 mg) for 20 hr at 110°. Excess of the reagent was decomposed with H_2O, filtered and then extracted with EtOAc. The solvent layer was evaporated to a residue (20 mg) which crystallized from MeOH to give **2a**, mp 260°. IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 2920, 1440, 1378, 1365, 1110, 1040, 910.

Acetylation of reduced product **2a**. The product **2a** (17 mg) with Ac_2O–pyridine at room temp. afforded a diacetyl derivative **2b** which crystallized from MeOH, mp 190°. IR ν_{max}^{KBr} cm^{-1}: 3480 (OH), 1740 (OAc). ^1H NMR: δ0.76, 0.83, 0.86, 0.88 (12H, quadruplet, 4Me), 1.06, 1.12 (3H each, *s*, 2Me), 1.96 (6H, *s*, 2-OCOMe), 4.08, 4.65 (1H each, *d*, *J* = 12 Hz, $-CH_2OAc$), 4.72 (1H, *m*, –CHOAc).

Dehydration of **2b**. The substance **2b** (12 mg) was refluxed in dry C_6H_6 containing freshly distilled BF_3-etherate (0.5 ml) for 1.5 hr. The reaction mixture was diluted with H_2O, extracted with EtOAc giving a residue (10 mg) which crystallized from MeOH to yield **3**, mp 180°. ^1H NMR: δ0.83, 0.86, 0.90, 0.93 (12H, quadruplet, 4Me), 1.1, 1.2 (3H each, *s*, 2Me), 1.96 (6H, *s*, 2-OCOMe), 3.9 (2H, AB*q*, *J* = 12 Hz, $-CH_2OAc$), 4.8 (1H, *m*, –CHOAc), 5.1 (1H, *br s*, –C=CH–). MS *m/z*: 512 $[M]^+$, 497, 452, 437, 392, 377, 328, 276, 216, 203 (base peak), 189, 187, 175, 161, 147, 133, 119, 107.

SeO_2 *oxidation of dehydrated product* **3**. Compound **3** (8 mg) in glacial AcOH containing freshly sublimed SeO_2 (12 mg) was refluxed for 2 hr. After the removal of AcOH *in vacuo*, the residue was extracted with Et_2O. The solvent layer yielded an amorphous powder. IR λ_{max}^{MeOH} nm: 243, 252 and 263.

Substance C (epicaltholide, **1b**). Colourless needles, mp 243–246°. IR ν_{max}^{KBr} cm^{-1}: 3250 (OH), 2900, 2850, 1742 (γ-lactone), 1450, 1440, 1380, 1360, 1220, 1180, 1120, 980, 920. ^1H NMR: δ0.78 (3H, *s*, Me), 0.81 (6H, *s*, 2Me), 1.1 (3H, *s*, Me), 1.15, 1.2 (3H each, *s*, 2Me), 3.72 (1H, *br s*, $W_{1/2}$ = 7 Hz, –CHOH). MS *m/z*: 442 $[M]^+$, 424 $[M - H_2O]^+$, 409 $[M - H_2O - Me]^+$, 235, 206 (base peak), 193, 189, 175 and 173.

Epicaltholide acetate. Epicaltholide (20 mg) was reacted overnight in pyridine and Ac_2O (0.4 ml each) at room temp. and worked up as usual. The residue was crystallized from MeOH, mp 209°. IR ν_{max}^{KBr} cm^{-1}: 1745 (γ-lactone), 1735 (OAc). ^1H NMR: δ0.80 (3H, *s*, Me), 0.86 (6H, *s*, 2Me), 0.93 (3H, *s*, Me), 1.14, 1.22 (3H, each, *s*, 2Me), 2.02 (3H, *s*, –OCOMe), 4.72 (1H, *br s*, $W_{1/2}$ = 7 Hz, –CHOAc). MS *m/z*: 484, 469, 424, 409, 248, 235, 189, 188, 175 and 173.

Acknowledgements—The authors thank Dr. Madhusudan, Mrs. K. Kapoor and Mr. Pati Ram for the spectral data. We are also

grateful to Prof. Dr. F. Bohlmann, Berlin, for 400 MHz ^{1}H NMR spectrum of caltholide acetate.

REFERENCES

1. Chopra, R. N., Nayar, S. L. and Chopra, I. C. (1956) *The Glossary of Indian Medicinal Plants*, p. 47. Council of Scientific and Industrial Research, New Delhi.
2. Drozd, G. A., Komissarenko, N. F. and Litvinenko, V. I. (1970) *Farm. Zh.* **25**, 57.
3. Khidasheli, V. D., Kiryanova, A. V. and Figurkin, B. A. (1976) *Nauch, Tr. Perm. Farmatsert. Int.* (*USSR*) 62.
4. Figurkin, B. A., Khidasheli, V. D., Pidemskii, E. L. and Goleneva, A. F. (1978) *Rastit. Resur.* **14**, 93.
5. Goleneva, A. F., Khidasheli, V. D. and Figurkin, B. A. (1979) *Rastit. Resur.* **15**, 115.
6. Setty, B. S., Kamboj, V. P. and Khanna, N. M. (1977) *Indian J. Exp. Biol.* **15**, 231.
7. Budzikiewicz, H., Wilson, J. M. and Djerassi, C. (1963) *J. Am. Chem. Soc.* **85**, 3688.
8. Corey, E. J. and Suggs, J. W. (1975) *Tetrahedron Letters* 2647.
9. Lehn, J.-M. and Ourisson, G. (1962) *Bull. Soc. Chim. Fr.* 1137.