Phytochemistry, Vol. 22, No. 9, pp. 1993–1995, 1983.
Printed in Great Britain.

0031-9422/83 $3.00+0.00

ISODEHYDROCOSTUS LACTONE AND ISOZALUZANIN C, TWO GUAIANOLIDES FROM *SAUSSUREA LAPPA*

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(*Revised received* 16 *November* 1982)

Key Word Index—*Saussurea lappa*; Compositae; sesquiterpene lactone; guaianolides; dehydrocostus lactone; eremanthine; estafiatin; zaluzanin C; isozaluzanin C; isodehydrocostus lactone.

Abstract—Two new guaianolides have been isolated from *Saussurea lappa* as minor components and have been named isodehydrocostus lactone and isozaluzanin C. A structure has been assigned to isodehydrocostus lactone on the basis of spectral data and correlation with estafiatin. The ^{1}H NMR data and dehydrogenation studies show that the other highly crystalline guaianolide is isomeric with zaluzanin C. Earlier 3β-H-zaluzanin C has however been reported to occur as a colourless oil.

INTRODUCTION

Extensive work has been published which describes the isolation and structure of constituents of costus root oil (*Saussurea lappa*). The Kashmir variety of *Saussurea lappa*, was studied by Govindan and Bhattacharyya [1]. They reported the isolation of α-cyclocostunolide, β-cyclocostunolide, alantolactone and isoalantolactone in addition to the two typical major components costunolide and dehydrocostus lactone. The co-existence of both the *cis* and *trans* types of lactones in this oil is of considerable biogenetic significance. In the continuation of our studies on terpenoids and their synthetic derivatives as a new group of plant growth regulators, two new guaianolides was isolated when we reinvestigated *Saussurea lappa* collected from the Kashmir region. In this communication we now report the structure and stereochemistry of these compounds.

RESULTS AND DISCUSSION

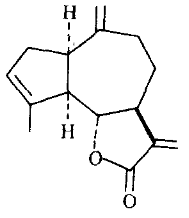
After the removal of costunolide and dehydrocostus lactone from the oil obtained by petrol extraction of the roots, the brown fragrant oil, on extentive chromatography and TLC on Si gel, resulted in the isolation of isodehydrocostus lactone (**1**) as a light green mobile liquid in the initial fractions, together with the previously known cyclocostunolides and alantolactones. Later fractions of these chromatographic separations resulted in the isolation of a new guaianolide, mp 143° (**6**).

Isodehydrocostus lactone (**1**) showed a characteristic violet spot on TLC obtained by spraying with 30% sulphuric acid followed by heating at 100°. Isodehydrocostus lactone readily reacted with diazomethane to afford its crystalline pyrazoline derivative (**9**), mp 110°. The IR and UV spectra of isodehydrocostus lactone showed the presence of an α, β-unsaturated γ-lactone $\nu_{\max}$ 1660, 1760 cm^{-1}; $\lambda_{\max}$ 206 nm, ε15 400), and both terminal ($\nu_{\max}$ 995 cm^{-1}) and trisubstituted ($\nu_{\max}$ 805 cm^{-1}) double bonds. The ^{1}H NMR spectrum of isodehydrocostus lactone had the two characteristic low field doublets (δ 5.4 and 6.2, $J = 3$ Hz) corresponding to the protons of an exocyclic methylene conjugated with the lactone carbonyl function. A singlet at δ 4.85 was attributed to the hydrogens of the exocyclic methylene group attached to C-10. In the case where this methylene group was attached at C-4 and thus would be close to an oxygen function, one proton would be deshielded to give the pattern which is invariably found in related guaianolides. No angular methyl groups were present, but an allylic methyl group (δ 1.75) was located on the trisubstituted double bond, whose sole proton absorbed as a broad doublet at δ 5.45.

Isodehydrocostus lactone (**1**) yielded chamazulene (**2**) on palladium dehydrogenation confirming that it was guaianolide. Catalytic hydrogenation of isodehydrocostus lactone resulted in the uptake of 3 mol of hydrogen to afford a hexahydro derivative, as a mixture of isomers (**3**), $C_{15}H_{24}O_2$, in which the γ-lactone moiety was retained ($\nu_{\max}$ 1775 cm^{-1}). The IR spectrum of the perhydro derivative (**3**) was superimposable on that of an authentic sample prepared from the hydrogenation of dehydrocostus lactone.

On the basis of the guaianolide carbon skeleton, the ethereal oxygen of the lactone must be located at C-6, since the hydrogen on this carbon showed only two *trans* diaxial couplings (with the C-5 and C-7 protons) appearing as a triplet centred at δ 4.05 ($J = 9$ Hz). The selective epoxidation of isodehydrocostus lactone with 1 mol of perbenzoic acid occurred at the trisubstituted double bond to afford **5**. The methyl absorption now occurred at δ 1.55 and the new epoxy-linked proton was seen as a singlet at δ 3.30 while the spectrum showed the presence of the intact terminal vinyl proton absorptions. These show that isodehydrocostus lactone is isomeric [2] with eremanthine (**4**) and the epoxide (**5**) derived from isodehydrocostus lactone then represents estafiatin. This identity was proved by its mp 103°, lit. [3] mp of estafiatin 104–106° and their superimposable ^{1}H NMR spectra [4].

After the conversion of isodehydrocostus lactone (**1**) to its pyrazoline (**9**) the C-6 proton showed an appreciable downfield shift (δ 4.75, *t*, $J = 9$ Hz). This proved that the $-N=N-$ grouping is *cis* placed with respect to the C-6



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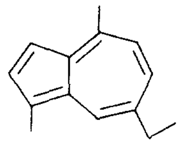

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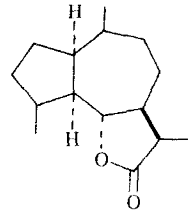

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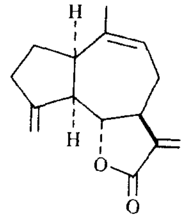

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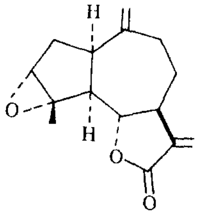

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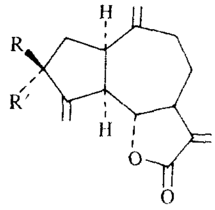
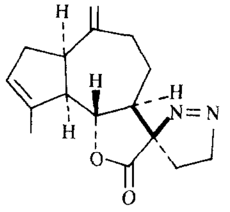


6	**7**	**8**
R = H	OH	>O
R' = OH	H	



9

proton and this trend has been found in all the related pyrazolines [5].

Isozaluzanin *C*(**6**) $C_{15}H_{18}O_3$, mp 143°, contained a free hydroxyl group ν_{max} 3550 cm^{-1} which could be readily acylated and oxidized to a ketone (**8**). The hydroxyl group of isozaluzanin C is, therefore, secondary. The remaining oxygens were present as part of a γ-lactone (ν_{max} 1760 cm^{-1}). The guaianolide nature was revealed by its dehydrogenation to yield chamazulene (**2**) in good yield. Ketone **8** was identified as dehydrozaluzanin C and, thus, established the structure of isozaluzanin C as **6**. A guaianolide isolated as a liquid has been previously [6] assigned structure **6**. The identity of the compound with mp 143° has also been confirmed as 3*β*-*H*-zaluzanin C by Bohlmann [personal communication]. Compound **6** isolated by us now is, however, highly crystalline with a 3α-configuration of the hydroxyl, since the 6*β*-hydrogen is less deshielded (δ 3.90) as compared with zaluzanin C (**7**, δ 4.14).

EXPERIMENTAL

All the compounds gave satisfactory C, H analysis. IR spectra were taken in Nujol suspensions. ^1H NMR spectra were recorded in $CDCl_3$ with TMS as int. standard. All the chromatographic separations were performed on Si gel.

Isodehydrocostus lactone (**1**) *and isozaluzanin C*(**6**). Powdered costus roots (Kashmir variety) were extracted with petrol (40–60°) at room temp. giving, after evaporation of the solvent, a light brown fragrant oil. This was stored at −4° for 2 days to remove most of the solid deposit consisting of costunolide and dehydrocostus lactone. The residue was chromatographed extensively on Si gel and appropriate fractions free from costunolide and dehydrocostus lactone eluted with petrol–Et_2O (19:1) were subjected to prep. TLC (10% $AgNO_3$–Si gel, C_6H_6–EtOAc, 19:1) giving **1** as a light green mobile liquid, $C_{15}H_{18}O_2$. (Found: C, 78.0; H, 7.92. $C_{15}H_{18}O_2$ requires: C, 78.23; H, 7.88%.) The tail fractions of the oil eluted with $CHCl_3$ after extensive prep. TLC afforded isozaluzanin C $C_{15}H_{18}O_3$, mp 143°. (Found: C, 73.24; H, 7.48. $C_{15}H_{18}O_3$ requires: C, 73.14; H, 7.37%.)

IR ν_{max}^{Nujol} cm^{-1}: OH 3600, γ-lactone 1760, methylenic double bonds 1660, 1640, 910 and 820. ^1H NMR: δ 3.90 (1H, *t*, *J* = 10 Hz, H-6), 4.68 (1H, *t*, *J* = 8 Hz, CHOH), 4.78 and 4.90 (1H each, *br s*, CH_2-10), 5.35 and 5.45 (1H each, *br s*, CH_2-4) 5.45 and 6.20 (two 1H, *d*, *J* = 3 Hz, CH_2-11).

Pyrazoline (**9**). A soln of **1** in Et_2O on reaction with excess CH_2N_2 gave pyrozoline (**9**), mp 110°, IR ν_{max}^{Nujol} cm^{-1}: 3050 (w), 1760 (s), 1640 (m) 1600 (m), 880 (s), 800 (m) ^1H NMR: δ 1.85 (3H, *br s*, allylic Me), 4.75 (3H, *t*, H-6 and $-CH_2-N=N-$), 4.87 (2H, *s*, CH_2-10) and 5.65 (1H, *br s*, H-3).

Dehydrogenation of isodehydrocostus lactone and isozaluzanin C. A mixture of isodehydrocostus lactone (300 mg) and Pd–C

10% (600 mg) in Nujol (5 ml) was heated at 300° for 30 min. The mixture was diluted with hexane and filtered. The deep blue filtrate was extracted with H_3PO_4 (5 ml). The acidic layer was separated, poured into crushed ice and re-extracted with hexane. The extract was dried, concd and passed over a bed of alumina. Its TNB adduct (50 mg) was prepared, which after several crystallizations from MeOH gave brownish-black needles mp 129° undepressed on admixture of an authentic sample. In a similar way compound **6** also afforded **2**.

Hydrogenation of isodehydrocostus lactone. A soln of isodehydrocostus lactone (200 mg) in EtOH (10 ml) was hydrogenated with PtO_2 (100 mg) until no more H_2 was absorbed. Filtration and evaporation of the solvent afforded a hexahydro derivative (**3**, 150 mg) whose IR spectrum was superimposable on that of an authentic sample of perhydro derivative prepared from dehydrocostus lactone.

Epoxidation of isodehydrocostus lactone. A soln of isodehydrocostus lactone (0.2 g) in $CHCl_3$ (5 ml) was reacted with 1 mol perbenzoic acid (2.0 ml 0.8 N perbenzoic acid in $CHCl_3$). After 12 hr at 9° the mixture was washed with aq. $NaHCO_3$, neutralized, dried (Na_2SO_4) and evaporated to afford a compound, mp 103° (0.18 g) identified as estafiatin (lit. [3] mp 104–106°). ^{1}H NMR: δ 1.56 (3H, *s*, Me-4), 3.30 (1H, *s*, H-3), 4.0 (1H, *dd*, $J = 9, 10$ Hz, H-6), 4.76 and 4.86 (1H, each *s*, CH_2-10), 5.4 and 6.1 (1H, each *d*, $J = 3$ Hz, CH_2-11). This ^{1}H NMR spectrum was identical to that of estafiatin [4].

Oxidation of isozaluzanin C. A soln of **6** (200 mg) in Me_2CO (10 ml) was reacted with 8 N CrO_3 in Me_2CO. The usual work-up afforded 150 mg **8**, mp 134° (lit. [7] mp 134–135°. ^{1}H NMR: δ 4.05 (1H, *t*, $J = 9$ Hz, H-6), 4.62 and 5.0 (1H each, *s*, CH_2-10), 5.60 and 6.40 (1H each, *d*, $J = 3$ Hz, CH_2-11), 5.90 and 6.30 (1H each, *d*, $J = 2$ Hz, CH_2-4). This ^{1}H NMR spectrum was identical with that reported [4].

Acknowledgements—We thank the Punjab State Government (India) for financial support. S.S. also thanks the Indian Council of Agricultural Research for financial support.

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