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GUAIANOLIDE FROM *SAUSSUREA LAPPA*

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Key Word Index—*Saussurea lappa*; Compositae; α-methylene-γ-lactone; biological activity; guaianolide; germacranolide; sesquiterpenoids; 12-methoxydihydrocostunolide.

Abstract—A new compound, 12-methoxydihydrodehydrocostuslactone, has been isolated from the essential oil of *Saussurea lappa*. The structure and stereochemistry were established by spectroscopic and chemical methods.

INTRODUCTION

The essential oil from costus roots (*Saussurea lappa* Clarke) is a rich source of two sesquiterpene lactones, dehydrocostuslactone (**1**) and costunolide (**2**) in addition to other minor components [1]. The biological activity of the oil is mainly attributed to the presence of these α-methylene-γ-lactones [2–4]. As a part of our continuing investigations of structure–biological activity relationships, we have isolated a new naturally occurring guaianolide, 12-methoxydihydrodehydrocostuslactone (**3**), from the essential oil.

RESULTS AND DISCUSSION

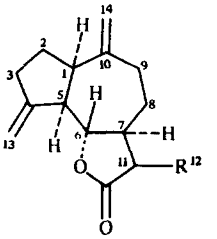
The new lactone (**3**), $C_{16}H_{22}O_3$ (HRMS $[M]^+$ 262.365), $[\alpha]_D^{20} + 19°$, exhibited ^{1}H NMR data characteristic of dehydrocostuslactone (**1**) and dihydrodehydrocostuslactone (**4**) (Table 1). The presence of an additional singlet for three hydrogens at $\delta 3.34$ suggested that one of the oxygen atoms was present as $-OCH_3$. This was supported by a mass ion peak at m/z 231 $[262-31]^+$. Its IR spectrum showed bands at 1780, 1030 (γ-lactone), 3080, 1640 and 890 (exomethylene double bond) cm^{-1}. All the above data suggested that the new compound was the guaianolide 12-methoxydihydrodehydrocostuslactone (**3**).

Further proof of the structure was obtained by the synthesis of **3** (IR and NMR) from dehydrocostuslactone upon treatment with methanol under alkaline conditions. In addition to this, it also established the stereochemistry at all centres except that of the 12-methoxy group. This reaction of dehydrocostuslactone is reversible under basic conditions and hence it should yield the thermodynamically more stable epimer at C-11. In *trans*-fused butanolides like santonin and related compounds the more stable configuration of the C-11 methyl is the one in which 12-methyl group is *trans* to the hydrogen at C-7. On the basis of this agreement [5], if it holds good in guaianolides also, the 12-methoxy group in compound (**3**) should have the β-configuration.

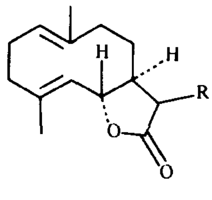
12-Methoxydihydrocostunolide (**6**) isolated earlier [6] from the essential oil was suspected to be an artefact produced from costunolide during isolation since methanol was used as the solvent for chromatographic elution. However, the isolation of the same compound in this study proves beyond doubt that this compound is a natural product. The presence of compounds **3** and **6** in the oil suggests that the parent lactones **1** and **2** might be present in bound form in the plant and that they migrate to other parts, especially in roots, of the plant as their 12-methoxy derivatives where they are again regenerated to perform various biological functions like root initiation and alleliopathogenicity. It has already been shown that lactones **1** and **2** are inhibitors of germination [2–4].

EXPERIMENTAL

Isolation of compound **3**. The essential oil (10 g), obtained by solvent extraction (hexane) from the powdered roots of *S. lappa*, was allowed to stand at 0° for several days when a solid (6.0 g)



1 R = $=CH_2$
3 R = $-CH_2OMe$
4 R = $-Me$



2 R = $=CH_2$
5 R = $-Me$
6 R = $-CH_2OMe$

Table 1. ^1H NMR data of compounds **1**, **3** and **4**

	1	**4**	**3**
C_6-H	3.96, *t*, $J = 8$ Hz	3.85, *t*, $J = 9$ Hz	3.81, *t*, $J = 9$ Hz
C-13 methylene	5.08, 5.28 ea. *s* (*br*)	5.35, 4.92 ea. *s* (*br*)	5.1, 5.19 ea. *s* (*br*)
C-14 methylene	4.92, 4.83 ea. *s* (*br*)	4.78, 4.72 ea. *s* (*br*)	4.76, 4.85 ea. *s* (*br*)
C-12 methylene	6.22, 4.47 ea. *d*, $J = 3$ Hz	1.14, *d*, $J = 7$ Hz	3.65, *d*, 2H, $J = 4$ Hz

separated out. After filtration the liquid was treated with an excess of an ethereal soln of CH_2N_2 and kept at 0° for 4 days for complete crystallization of solid pyrazolines (1.2 g). The concentrated filtrate (3.0 g) was subjected to CC over $AgNO_3$–silica gell (1:10, 300 g). Elution of the column with hexane–Et_2O (9:1) gave dihydrodehydrocostuslactone (**4**, 610 mg) followed by 12-methoxydihydrodehydrocostuslactone (**3**, 350 mg). Compound **3** showed $[\alpha]_D^{20} + 19°$ HRMS ($[M]^+$ 262.365), $C_{16}H_{22}O_3$ requires 262.349; IR ν_{max}^{film} cm^{-1}: 3090, 1780, 1640, 1455, 1030, 1005, 890; ^1H NMR ($CDCl_3$, 60 MHz): δ3.34 (*s*, 3H), 3.65 (*d*, 2H, $J = 4$ Hz), 4.76 and 4.85 [*s* (*br*), 1H each] and 5.1 and 5.19 [*s* (*br*) 1H each], 3.81 (*t*, $J = 9$ Hz). Further elution of the column with hexane–Et_2O (4:1) gave pure dihydrocostunolide (**5**, 480 mg) followed by compound **6** (210 mg) [6].

Conversion of dehydrocostuslactone (**1**) *to* **3**. To a soln of dehydrocostuslactone (1500 mg) in MeOH (10 ml) was added KOH (300 mg) dissolved in MeOH (5 ml). After keeping the mixture overnight at room temp., it was diluted with cold H_2O and extracted with Et_2O. The Et_2O extracts yielded the 12-methoxy derivative (320 mg), $[\alpha]_D^{20} + 19°$ and IR and NMR superimposable on those of compound 3 isolated from the costus root oil.

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