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DEHYDROCOSTUSLACTONE AND PLANT GROWTH ACTIVITY OF DERIVED GUAIANOLIDES

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Key Word Index—*Saussurea lappa*; Compositae; root promoters; sesquiterpene lactone; guaianolide; dehydrocostuslactone; estafiatin; isozaluzanin C; isodehydrocostuslactone.

Abstract—The stereostructures of two new guaianolides, isodehydrocostuslactone and isozaluzanin C, isolated previously from *Saussurea lappa*, have been confirmed by their correlation with dehydrocostuslactone. Twenty new derivatives have been synthesized from these guaianolides and these have been tested as plant growth regulators. The conjugated lactones which have an exocyclic methylene group at C-4, conjugated with a C-3 ketone, show distinct enhancement in their root-forming potential, as compared with their 3-deoxy derivatives. Of further significance is the fact that these ketones display maximum activity only at lower concentrations. Other compounds show the expected structure–biological activity relationships displayed in general by guaianolides. However, the presence of an epoxide at the C-3, C-4 position does not affect the biological activity, which is indeed the case when the epoxide group occupies the C-4, C-14 position in guaianolides. The major biological parameter studied was rooting in-stem cuttings of *Phaseolus aureus*.

INTRODUCTION

Stereostructures **4** and **5** were assigned respectively to isodehydrocostuslactone and isozaluzanin C [1], the two new guaianolides isolated from *Saussurea lappa*. We have now confirmed these stereostructures further by the conversion of dehydrocostuslactone (**6**) of known stereostructure to compounds **4** and **5**. In order to provide an opportunity to establish whether the structure and plant growth activity relationships documented earlier in the case of guaianolides hold good, we have now synthesized 20 guaianolides from the naturally occurring terpenoid lactones isodehydrocostustulactone (**4**) and isozaluzanin C (**5**) and have evaluated their root-forming potential.

RESULTS AND DISCUSSION

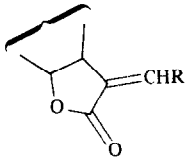
Oxidation with SeO_2 *and* tert-*butyl hydroperoxide (TBHP)*

Isozaluzanin C (**5**) was prepared from dehydrocostuslactone (**6**) by carrying out an allylic oxidation with SeO_2 in combination with *tert*-butyl hydroperoxide (TBHP) [2]. In dehydrocostuslactone, several points of attack are available. However, this reaction under mild and controlled conditions afforded a compound (mp 143°) in almost quantitative yield, identical in all respects (IR spectra and mmp determination) to isozaluzanin C (**5**) [1]. This specific allylic oxidation only at the C-3 position is an interesting aspect of this reaction. On further oxidation with SeO_2 and *tert*-butyl hydroperoxide for a longer period, isozaluzanin C (**5**) afforded a compound (mp 166°) in quantitative yield. The C-6 hydrogen of this compound displayed in its ^1H NMR spectrum only one *trans*-diaxial coupling (with the C-7 proton) appearing as a doublet centred at $\delta 3.86$ ($J = 9$ Hz). This confirmed the position of the second hydroxyl group at C-5, and indicated structure **7** for the compound of mp 166°. In confirmation with this assignment, this compound on acetylation afforded a hydroxy-acetate (**21**). In this oxidation, therefore, the second hydroxyl group is added at the C-5 position, leaving the other allylic positions (i.e. C-1 and C-9) totally unattacked. In confirmation with these results, compounds **23** and **27** also underwent oxidation with SeO_2 and *tert*-butyl hydroperoxide exclusively at the C-3 position to afford **24** and **28**, respectively. The α-stereochemistry of the C-3 hydroxyl group in **24** and **28** was proved by converting isozaluzanin C (**5**) to its pyrazoline derivative (**22**). In this derivative, the C-6 proton showed an appreciable downfield shift, compared with the parent compound isozaluzanin C (**5**). This showed that the –N=N– grouping is *cis*-placed with respect to the C-6 proton. On pyrolysis, this pyrazoline afforded a two-component mixture, which was separated by chromatography to yield compounds with mp 96° and 110°, and these were completely identical (superimposable IR spectra and mmp determination) to **28** and **24**, respectively. Similarly, compound **31** on oxidation afforded compound **32**.

Significantly, a similar reaction on isodehydrocostuslactone (**4**) led to oxidation at the C-1 allylic position to afford **16** in almost quantitative yield. In the case of the oxidation of isodehydrocostuslactone, the otherwise expected oxidation at the methylene group allylic to the double bond does not occur [3]. To confirm these findings, isodehydrocostuslactone-derived C_{16}-guaianolides (**9** and **10**) on oxidation with SeO_2 and *tert*-butyl hydroperoxide also led to the formation of **18** and **17**, respectively, involving specifically allylic oxidation at the C-1 position.

Oxidation with Jones' chromic acid reagent

The hydroxy compounds **5**, **24** and **28** on Jones'



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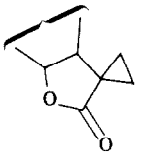
2 R = Me



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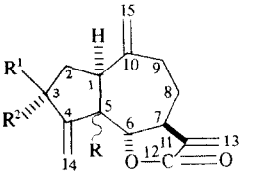
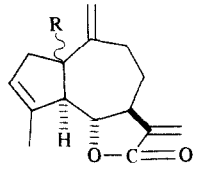
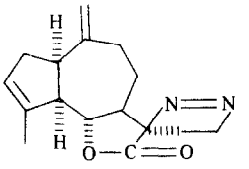


	5	**6**	**7**	**19**	**20**	**21**
R	αH	αH	OH	αH	αH	OH
R^1	H	H	H	H	>O	H
R^2	OH	H	OH	OAc		OAc



	4	**16**
R	αH	OH



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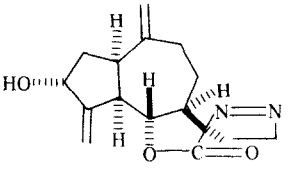

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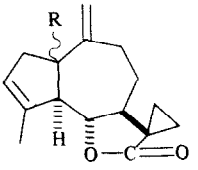
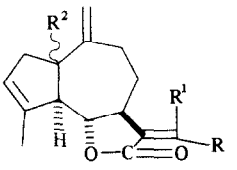
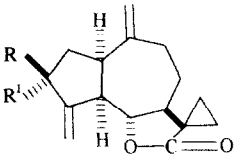


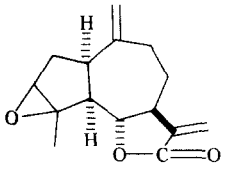
	9	**18**
R	αH	OH



	10	**11**	**17**
R	Me	H	Me
R^1	H	Me	H
R^2	αH	αH	OH



	23	**24**	**25**	**26**
R	H	H	H	>O
R^1	H	OH	OAc	



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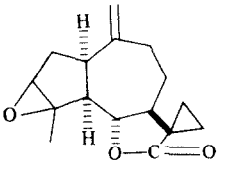

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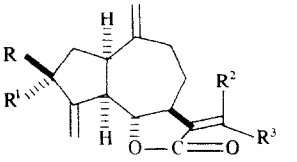
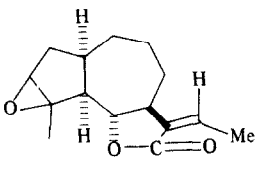


	27	**28**	**29**	**30**	**31**	**32**
R	H	H	H	>O	H	H
R^1	H	OH	OAc		H	OH
R^2	H	H	H	H	Me	Me
R^3	Me	Me	Me	Me	H	H



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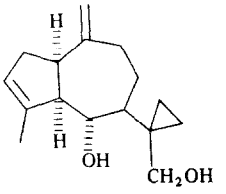

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chromic acid oxidation afforded the corresponding compounds **20** (mp 134°), **26** (mp 118°) and **30** (mp 116°), respectively. These oxidations could also be carried out by active mangenese dioxide. The ^{1}H NMR and IR spectral data (Table 1) are sufficient to assign these structures.

Table 1. IR and ^{1}H NMR spectral data for guaianolides

Compound	IR (cm^{-1})	^{1}H NMR* H-13	H-6	H-3	H-14	H-15	H-16	H-12	–OAc
7	3440, 1755, 1670, 1640, 905, 820	5.54, 6.13 (1H each, *d*) *J* = 3	3.86 (1H, *d*) *J* = 9	4.02 (1H, *t*) *J* = 8	5.47, 5.62 (1H, *d*) *J* = 2	4.88, 4.95 (1H, *br s*)	—	—*	—
19	1760, 1735, 1255, 900	5.53, 6.20 (1H each, *d*) *J* = 3	4.10 (1H, *t*) *J* = 9	5.60 (1H, *t*) *J* = 9	5.30, 5.50 (1H each, *br s*)	5.00 (2H, *br s*)	—	—*	2.15 (3H, *s*)
21	3220, 1760, 1720, 1630, 1250, 910	5.61, 6.30 (1H each, *d*) *J* = 3	4.20 (1H, *d*) *J* = 9	5.22 (1H, *t*) *J* = 9	5.55, 5.85 (1H each, *br s*)	5.10, 4.90 (1H each, *s*)	—	—	2.20 (3H, *s*)
22	3400, 1770, 1640, 900	—	4.62 (1H, *t*) *J* = 9	4.48 (1H, *t*) *J* = 8	5.48, 5.60 (1H each, *br s*)	4.72 (2H, *br s*)	4.88 (2H, *t*) *J* = 9	—	—
24	3500, 1750, 1640, 900	0.6–1.1 (*m*)	3.95 (1H, *t*) *J* = 9	4.65 (1H, *t*) *J* = 9	5.29, 5.40 (1H each, *br s*)	4.7, 4.85 (1H each, *br s*)	0.6–1.1 (*m*)	—	—
25	1760, 1620, 1220, 1150, 900	0.6–1.1 (*m*)	4.0 (1H, *t*) *J* = 9	5.68 (1H, *t*) *J* = 8	5.35, 5.55 (1H each, *br s*)	4.80, 4.92 (1H each, *br s*)	0.6–1.1 (*m*)	—	2.08 (3H, *s*)
26	1760, 1720, 1623, 885, 825	0.5–1.0 (*m*)	4.05 (1H, *t*) *J* = 9	—	5.8, 6.2 (1H each, *br s*)	4.51, 4.85 (1H each, *s*)	0.6–1.1 (*m*)	—	—
28	3420, 1760, 1650, 1630, 890, 860	6.1 (1H, *dq*) *J* = 3, 8	3.88 (1H, *t*) *J* = 9	4.69 (1H, *t*) *J* = 8	5.35, 5.5 (1H each, *s*)	4.77, 4.90 (1H each, *s*)	2.24 (3H, *dd*) *J* = 3, 8	—	—
29	1750, 1660, 1250, 900, 860	6.22 (1H, *dq*) *J* = 3, 8	3.94 (1H, *t*) *J* = 8	5.78 (1H, *t*) *J* = 8	5.49, 5.64 (1H each, *br s*)	4.88, 5.00 (1H each, *br s*)	2.28 (3H, *dd*) *J* = 3, 8	—	2.12 (3H, *s*)
30	1750, 1725, 1650, 900, 860	6.40 (1H, *dq*) *J* = 3, 8	3.96 (1H, *t*) *J* = 9	—	5.95, 6.30 (1H each, *br s*)	4.65, 5.02 (1H each, *br s*)	2.30 (3H, *dd*) *J* = 3, 8	—	—
32	3500, 1760, 1680, 1660, 900	6.83 (1H, *dq*) *J* = 3, 8	3.98 (1H, *t*) *J* = 9	4.70 (1H, *t*) *J* = 7	4.88, 5.32 (1H, *d*) *J* = 3	4.92, 5.5 (1H each, *br s*)	1.96 (3H, *dd*) *J* = 3, 8	—	—

*Coupling constants (*J*) in Hz.

Acetylation of compounds **5**, **7**, **24** *and* **28**

The acetylations of compounds **5**, **7**, **24** and **28** were carried out with acetic anhydride and pyridine. As expected, in the esters the CHOAc proton showed a downfield shift of around δ1.0.

Isodehydrocostuslactone and its derivatives

In order to convert dehydrocostuslactone (**6**) to isodehydrocostuslactone (**4**), the exocyclic double bond at C-4 had to be isomerized to the $\Delta^{3(4)}$-position. This type of isomerization has been reported previously [4] on dehydrocostuslactone by using an excess of BF_3-etherate, when both the *exo*-double bonds were isomerized to *endo*-positions. During the present investigations, we selectively isomerized the C-4 exocyclic double bond to the $\Delta^{3(4)}$-position in *ca* 70% yield by refluxing a solution of dehydrocostuslactone in benzene with iodine. The isomerized product was found to be identical in all respects to the naturally occurring [1] isodehydrocostuslactone (**4**).

The pyrazoline (**8**), prepared [1] by the reaction of diazomethane on **4**, upon pyrolysis furnished, as usual, a mixture of two isomeric components (mp 42° and 49°), which were separated by column chromatography and were identified as **9** and **10**, respectively, by their ^{1}H NMR spectral data (Table 2).

Reaction of compound **10** with diethylamine afforded a mixture of two products, one of which was shown to be the starting compound (TLC and mmp); the other product (mp 58°) showed its trisubstituted olefinic proton as a doublet of quartets at δ6.9. This required the compound of mp 58° to be the *E*-isomer (**11**). The selective epoxidation of compounds **9** and **10** with *m*-chloroperbenzoic acid occurred at the trisubstituted double bond to afford the monoepoxides **13** and **14**, in which the methyl absorption occurred at δ1.55 and 1.65 compared to δ1.8 in the parent compounds, and the new epoxy-linked protons were seen as singlets at δ3.25 and 3.20. The epoxides **13** and **14** showed in their ^{1}H NMR spectra the presence of the intact terminal vinyl proton absorptions and also an overall resemblance with the ^{1}H NMR spectral features of estafiatin (**12**) [1].

Biological activity

Recent publications from our laboratory have established that among terpenoid lactones and their synthetic derivatives, guaianolides rank high to display well-correlated structure–biological activity relationships [5]. A variety of γ-lactones in which a trisubstituted double bond (**2**) or a cyclopropane ring (**3**) is conjugated with the lactone carbonyl are more active [5] than their parent (**1**) α-methylene-γ-lactones [6]. It has been further established that only in the case of guaianolides does the presence of a C-4 epoxy or a C-4, C-10 ether group further enhance the biological activity [7] associated with the parent lactone. A similar effect of the epoxy group was not observed in the case of eudesmanolides [5]. Moreover, the presence of a hydroxyl group at C-4 or C-10 and an epoxy at C-10 had no such effect [7].

In order to study the effect of oxygen-containing groups at other positions on the guaianolide skeleton, we synthesized and evaluated the biological activity of 20 guaianolide derivatives. The significant results are given below.

Biological evaluation of compounds **20**, **26** and **30** (Table 3) indicated that the introduction of a keto group at C-3 leads to an enhancement in root formation over their three deoxy compounds **6**, **23** and **27**, respectively. Another significant point is that compounds **20**, **26** and **30** showed maximum activity at lower concentrations while with the higher concentrations at which their deoxy compounds displayed maximum activity these compounds became toxic. In compounds **20**, **26** and **30**, the introduction of the keto group led to the introduction of an α,β-unsaturated ketone moiety. It has been shown previously that an α,β-unsaturated ketone moiety confers root formation activity in a variety of terpenoids [8]. Thus in compounds **20**, **26** and **30**, there are two active moieties, i.e. a conjugated γ-lactone and an α,β-unsaturated ketone, so the enhanced activity displayed by these compounds may be due to an additive effect.

In confirmation with earlier reports [7], the present data also show that the presence of a hydroxyl group at the C-1,C-3 or C-5 position lowers, to some extent, the plant growth activity associated with their parent conjugated γ-lactones, while C-3 acetyl derivatives lead to slight enhancement of biological activity as compared with C-3 hydroxyl derivatives.

Reference to Table 3 clearly shows that, unlike the presence of an epoxy ring at the C-4,C-14 position, which had earlier been shown to enhance the biological activity [7], the presence of an epoxide group at the C-3,C-4 position lowers it. Thus the epoxides **12**, **13** and **14** are distinctly less active compared with their parent lactones (**4**, **9** and **10**). Compounds **9** and **10**, as expected [5], are much more potent than their parent α-methylene-γ-lactone compound (**4**). Another observation worth noting is that the *E*-isomer (**11**) displays almost half the activity compared with its *Z*-isomer (**10**). This pattern of a definite change in biological activity occurring with *E* to *Z* isomerization in the case of terpenoids was observed without any exceptions.

EXPERIMENTAL

General procedures. The general procedure used for the work-up of the reaction mixtures consisted of the addition of cold H_2O, extraction with Et_2O, neutralization of the extracts, and drying over Na_2SO_4. After removal of the solvent, the different products were separated by CC on silica gel. IR spectra were taken in nujol suspensions. ^{1}H NMR spectra were recorded in $CDCl_3$, with TMS as the internal standard.

Oxidation with SeO_2 *and* tert-*butyl hydroperoxide (TBHP).* A soln of the lactone (1.0 g) in CH_2Cl_2 (5 ml) was added dropwise to a previously prepared soln of SeO_2 (0.2 g) in CH_2Cl_2 (10 ml) and 90% TBHP (2 ml). After 2 hr at room temp., the reaction was complete.

Epoxidation with m-*chloroperbenzoic acid.* To a soln of the lactone (0.5 g) in CH_2Cl_2 (10 ml) was added *m*-chloroperbenzoic acid (*ca* 85% purity, 0.5 g). After stirring for 2 hr at 5°, the product mixture was isolated; the monoepoxide was the major component.

Oxidation with Jones' reagent. This oxidation was carried out as usual by dissolving the compound (0.5 g) in Me_2CO (10 ml) and adding 8 N CrO_3 at 5° until the persistence of an orange colour (1 hr).

Acetylations. Acetylation was carried out by dissolving the compound (0.5 g) in pyridine (5 ml) and adding Ac_2O (0.5 ml). After 12 hr at room temp., the acetylation was complete in each case.

Table 2. IR and ^{1}H NMR spectral data for guaianolides

Compound	IR (cm^{-1})	^{1}H NMR* H-13	H-6	H-3	H-14	H-15	H-16	H-12	–OAc
9	1760, 1660, 1640, 880, 830	0.6–1.3 (4H, *m*)	4.5 (1H, *t*) $J = 9$	5.55 (1H, *m*)	1.8 (3H, *br s*)	4.8 (2H, *s*)	0.6–1.3 (4H, *m*)	—	—
10	1760, 1660, 1640, 892, 850	6.2 (1H, *dq*) $J = 3, 9$	4.0 (1H, *t*) $J = 9$	5.50 (1H, *m*)	1.85 (3H, *br s*)	4.85 (2H, *s*)	2.20 (3H, *dd*) $J = 3, 9$	—	—
11	1760, 1690, 1655, 892, 860	6.9 (1H, *dq*) $J = 3, 9$	4.2 (1H, *dd*) $J = 9, 11$	5.60 (1H, *m*)	1.80 (3H, *br s*)	4.95 (2H, *s*)	2.10 (3H, *dd*) $J = 3, 9$	—	—
13	1750, 1640, 892	0.7–1.2 (4H, *m*)	4.1 (1H, *t*) $J = 9$	3.25 (1H, *s*)	1.55 (3H, *br s*)	4.75, 4.85 (1H each, *br s*)	0.7–1.2 (4H, *m*)	—	—
14	1750, 1660, 1640, 880, 820	6.2 (1H, *dq*) $J = 3, 9$	4.0 (1H, *dd*) $J = 9, 11$	3.30 (1H, *s*)	1.65 (3H, *br s*)	4.75, 4.85 (1H each, *br s*)	2.10 (3H, *dd*) $J = 3, 9$	—	—
15	3250, 1640, 880, 800	0.5–1.0 (*m*)	4.12 (1H, *t*) $J = 9$	5.55 (1H, *br m*)	2.30 (3H, *br s*)	5.14, 5.22 (1H each, *br s*)	0.5–1.0 (*m*)	ABq $\delta A = 4.0$ $\delta B = 4.7$ $J = 12$	—
16	3260, 1750 892	5.36, 6.10 (1H each, *d*) $J = 3$	3.8 (1H, *t*) $J = 9$	5.5 (1H, *br m*)	1.82 (3H, *br s*)	4.95, 5.05 (1H, *s*)	—	—	—
17	3350, 1740, 1660, 910, 860	6.05 (1H, *dq*) $J = 3, 9$	3.8 (1H, *t*) $J = 9$	5.45 (1H, *br s*)	1.88 (3H, *br s*)	4.98, 5.10 (1H each, *s*)	2.12 (3H, *dd*) $J = 3, 9$	—	—
18	3320, 1760 900, 830	0.8–1.2 (*m*)	3.91 (1H, *t*) $J = 9$	5.45 (1H, *br m*)	1.85 (3H, *br s*)	4.95, 5.05 (1H each, *s*)	0.8–1.2 (*m*)	—	—

*Coupling constants (J) in Hz.

Table 3. Effects of 3, 5, 10, 15 and 20 mg/l. of guaianolides on the number of roots per rooted segment produced by hypocotyl cuttings of *P. aureus* after 7 days

Compound	Number of roots*				
	3	5	10	15	20
4	—	9.3 ± 2.1	11.7 ± 1.9	14.5 ± 1.8	20.8 ± 2.0
5	—	7.4 ± 2.3	9.6 ± 0.5	13.0 ± 1.9	Toxic
6	—	10.1 ± 1.2	14.2 ± 2.6	17.0 ± 1.7	20.1 ± 2.2
7	—	7.0 ± 2.1	8.4 ± 0.9	10.7 ± 2.2	11.4 ± 2.6
9	—	10.2 ± 1.7	12.0 ± 1.0	16.0 ± 1.2	P
10	—	12.7 ± 1.7	20.1 ± 2.0	22.0 ± 2.5	26.0 ± 4.0
11	—	8.4 ± 2.3	12.0 ± 2.7	14.0 ± 2.0	13.2 ± 1.7
12	—	8.9 ± 1.2	9.2 ± 1.6	9.8 ± 1.4	10.6 ± 1.6
13	—	8.2 ± 1.8	9.7 ± 0.5	10.2 ± 1.7	13.6 ± 2.4
14	—	10.0 ± 1.6	19.8 ± 2.8	14.8 ± 2.2	10.3 ± 2.0
16	—	9.8 ± 1.3	13.8 ± 1.9	10.6 ± 1.4	P
17	—	6.2 ± 1.9	16.0 ± 1.8	10.2 ± 1.9	12.9 ± 2.3
18	—	10.5 ± 1.1	11.9 ± 1.1	14.1 ± 1.6	10.9 ± 1.2
19	—	7.6 ± 1.2	9.3 ± 0.8	14.2 ± 2.1	14.1 ± 1.4
20	20.5 ± 1.8	19.0 ± 1.8	Toxic	Toxic	Toxic
21	—	6.6 ± 1.2	8.3 ± 1.6	12.1 ± 2.3	13.1 ± 2.3
23	—	5.3 ± 1.4	14.6 ± 2.8	17.9 ± 1.8	21.7 ± 2.8
24	—	6.8 ± 0.3	8.4 ± 0.6	7.8 ± 0.3	8.2 ± 0.9
26	15.5 ± 2.1	16.0 ± 2.1	18.8 ± 0.8	P	Toxic
27	—	6.0 ± 2.1	14.6 ± 2.6	18.8 ± 2.7	23.0 ± 2.2
28	—	7.0 ± 1.2	10.2 ± 0.8	10.0 ± 2.0	8.0 ± 1.8
29	—	8.0 ± 1.4	8.5 ± 0.9	14.2 ± 1.5	12.4 ± 1.6
30	16.4 ± 2.4	24.2 ± 2.4	27.0 ± 3.8	Toxic	Toxic
32	—	7.4 ± 0.9	7.5 ± 1.2	10.8 ± 1.6	14.8 ± 1.3

*Control experiment, water = 7.1 ± 1.3.
P = Primordias.

Formation of pyrazolines and their pyrolysis. To a soln of the lactone in Et_2O was added CH_2N_2 in Et_2O until the reaction was complete (TLC). The pyrazoline thus obtained was heated at a temp. 20° above its mp.

Oxidation of dehydrocostuslactone (**6**) *with* SeO_2 *and* tert-*butyl hydroperoxide.* Compound **6** was reacted with SeO_2 and *tert*-butyl hydroperoxide and the product mixture was chromatographed to furnish **5**, mp 143°, upon elution with petrol–Et_2O (7:3), identical in all respects to naturally occurring isozaluzanin C. Further elution of the column with petrol–Et_2O (1:1) afforded pure crystalline compound **7**, mp 166°. (Found: C, 68.60; H, 6.80. $C_{15}H_{18}O_4$ requires: C, 68.68; H, 6.92%.) Oxidation of **5** with SeO_2 and TBHP afforded **7** in quantitative yield.

Reaction of compound **23** *with* SeO_2 *and* tert-*butyl hydroperoxide.* Compound **23** was treated with SeO_2 and *tert*-butyl hydroperoxide. The product mixture was chromatographed to yield **24**, mp 110°, as a major component upon elution with petrol–Et_2O (7:3). (Found: C, 73.70; H, 7.60. $C_{16}H_{20}O_3$ requires: C, 73.82; H, 7.74%.)

Reaction of compound **27** *with* SeO_2 *and* tert-*butyl hydroperoxide.* Compound **27** was treated with SeO_2 and *tert*-butyl hydroperoxide. The product mixture was chromatographed to afford **28**, mp 96°, as a major component upon elution with petrol–Et_2O (7:3). (Found: C, 73.90; H, 7.60. $C_{16}H_{20}O_3$ requires: C, 73.82; H, 7.74%.)

Reaction of compound **31** *with* SeO_2 *and* tert-*butyl hydroperoxide.* Compound **31** on treatment with SeO_2 and *tert*-butyl hydroperoxide afforded a mixture, which on chromatography yielded **32** as a yellow, mobile liquid upon elution with petrol–Et_2O (7:3). (Found: C, 73.93; H, 7.60. $C_{16}H_{20}O_3$ requires: C, 73.82; H, 7.74%.)

Pyrazoline (**22**). A soln of **5** in Et_2O on reaction with excess CH_2N_2 gave pyrazoline **22**, mp 127°. (Found: C, 66.58; H, 6.90. $C_{16}H_{20}O_3N_2$ requires: C, 66.64; H, 6.99%.) Pyrolysis of **22** at 147° for 1 hr afforded a two-component mixture, which on chromatography yielded **28** upon elution with petrol–Et_2O (7:3). Further elution of the column with the same solvent afforded pure **24**.

Jones' oxidation of compounds **23** *and* **27**. Jones' oxidation of compounds **23** and **27** gave the corresponding compounds **26**, mp 118° (found: C, 74.00; H, 6.93; $C_{16}H_{18}O_3$ requires: C, 74.39; H, 7.02%) and **30**, mp 116° (found: C, 74.10; H, 6.95; $C_{16}H_{18}O_3$ requires: C, 74.39; H, 7.02%).

Acetylation of compounds **5**, **7**, **24** and **28**. Acetylation of **7** afforded a crystalline compound **21**, mp 103°. (Found: C, 66.98; H, 6.50. $C_{17}H_{20}O_5$ requires: C, 67.09; H, 6.62%.) Similarly, acetylation of **5**, **24** and **28** yielded the compounds **19** (found: C, 70.00; H, 6.88; $C_{17}H_{20}O_4$ requires: C, 70.81; H, 6.99%), **25** (found: C, 71.00; H, 7.05; $C_{18}H_{22}O_4$ requires: C, 71.50; H, 7.33%) and **29** (found: C, 70.98; H, 7.10; $C_{18}H_{22}O_4$ requires: C, 71.50; H, 7.33%), respectively.

Reaction of dehydrocostuslactone (**6**) *with* I_2. To a soln of **6** (5 g) in dry C_6H_6 (100 ml) was added I_2 (0.2 g). After refluxing for 2 hr, the mixture was washed with aq. $Na_2S_2O_7$ and the product mixture isolated in the usual way was chromatographed. Elution of the column with petrol–Et_2O (30:1) afforded isodehydrocostuslactone as a yellow, mobile liquid, identical in all respects to the naturally occurring isodehydrocostuslactone.

Pyrolysis of compound **8**. The pyrazoline **8** (4 g) on pyrolysis at 130° for 1 hr afforded a two-component mixture, which on chromatography yielded **10**, mp 49°, upon elution with petrol–Et_2O (30:1). (Found: C, 78.50; H, 8.10. $C_{16}H_{20}O_2$ requires: C, 78.65; H, 8.25%.) Further elution with petrol–Et_2O (19:1) afforded pure **9**, mp 42°. (Found: C, 78.75; H, 8.35. $C_{16}H_{20}O_2$ requires: C, 78.65; H, 8.25%.)

Epoxidation of compounds **9** *and* **10**. Epoxidation of **9** was carried out with *m*-chloroperbenzoic acid. On work-up, elution of the column with petrol–Et_2O (19:1) yielded **9**. Further elution with petrol–Et_2O (9:1) afforded pure compound **13**. (Found: C, 73.90; H, 7.60. $C_{16}H_{20}O_3$ requires: C, 73.82; H, 7.74%.) Similarly, epoxidation of **10** afforded **14**, mp 94°. (Found: C, 73.70; H, 7.65. $C_{16}H_{20}O_3$ requires: C, 73.82; H, 7.74%.)

Isomerization of compound **10**. Compound **10** (1 g) in EtOH (150 ml) was stirred with diethylamine (5 ml) at 50° for 12 hr. The product mixture was chromatographed to furnish **10**, upon elution with petrol–Et_2O (30:1). Further elution with petrol–Et_2O (19:1) afforded its isomer **11**, mp 58°. (Found: C, 78.70; H, 8.30. $C_{16}H_{20}O_2$ requires: C, 78.65; H, 8.25%.)

Oxidation of **4** *with* SeO_2 *and* tert-*butyl hydroperoxide*. Compound **4** (0.5 g) was treated with SeO_2 and *tert*-butyl hydroperoxide. The product mixture was chromatographed to yield **4** on elution with petrol–Et_2O (30:1). Further elution with petrol–Et_2O (9:1) afforded **16**, mp 102°. (Found: C, 73.20; H, 7.40. $C_{15}H_{18}O_3$ requires: C, 73.14; H, 7.37%.)

Reaction of compound **9** *with* SeO_2 *and* tert-*butyl hydroperoxide*. Compound **9** was reacted with SeO_2 and *tert*-butyl hydroperoxide and the product mixture was chromatographed to give **9** on elution with petrol–Et_2O (30:1). Further elution of the column with petrol–Et_2O (9:1) yielded **18**, mp 163°. (Found: C, 73.93; H, 7.85. $C_{16}H_{20}O_3$ requires: C, 73.82; H, 7.74%.)

Reaction of compound **10** *with* SeO_2 *and* tert-*butyl hydroperoxide*. Compound **10** was treated with SeO_2 and *tert*-butyl hydroperoxide and the product mixture was chromatographed to furnish **10** upon elution with petrol–Et_2O (30:1). Further elution with petrol–Et_2O (9:1) gave **17**, mp 108°. (Found: C, 73.90; H, 7.85. $C_{16}H_{20}O_3$ requires: C, 73.82; H, 7.74%.)

Reduction of compound **9** *with* $LiAlH_4$. A soln of **9** (1.0 g) in dry Et_2O (20 ml) was added to a slurry of $LiAlH_4$ (0.4 g) in dry Et_2O (20 ml). After 1 hr of reflux, compound **15**, mp 105°, was isolated in quantitative yield. (Found: C, 77.50; H, 9.70. $C_{16}H_{24}O_2$ requires: C, 77.37; H, 9.74%.)

Biological testing. For the root initiation study on hypocotyl cuttings of *Phaseolus aureus*, seedlings were grown under continuous illumination. When the hypocotyls were 5–6 cm long, cuttings were made by excision 4 cm below the cotyledonary node, leaving the cotyledonary leaves and apex intact. In all, five concns (3, 5, 10, 15 and 20 mg/l.), together with water as control, were tested. For all treatments, 10 replicates were cultured in vials each containing 30 ml test soln. Final observations were recorded on day 8. The experiment was repeated three times at $27 \pm 2°$.

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