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CHEMISTRY OF COSTUNOLIDE AND BIOLOGICAL ACTIVITY OF THE DERIVED LACTONES

P. S. KALSI, SARITA KHURANA and K. K. TALWAR

Department of Chemistry, Punjab Agricultural University, Ludhiana, India

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Key Word Index—Terpenoid lactones; root promoters; germacranolides; melampolides; eudesmanolides.

Abstract—Costunolide and its derived C-16 germacranolides on oxidation with selenium dioxide–*t*-butyl hydroperoxide afforded two melampolides, an aldehydolactone and the corresponding hydroxylactone, in each case. Structures were assigned to these melampolides on the basis of spectral data and chemical correlation. The aldehydolactones were significantly more active root promotors than their parent lactones. Costunolide and related germacranolides underwent cyclization on treatment with iodine and pyridinium chlorochromate to afford interesting products. (−)-β-Frullanolide has been synthesized and shown to be biologically more active when compared with its parent *trans*-lactone.

INTRODUCTION

Work on the chemical transformations of costunolide and related germacrane derivatives occupies an important place in the history of cyclodecadiene chemistry. We now report on (a) the oxidation of the germacranolides with selenium dioxide–*t*-butyl hydroperoxide (TBHP) and (b) the cyclization of the germacranolide skeleton with pyridinium chlorochromate [1] and iodine. The synthesis of (−)-β-frullanolide (**31**) [2] has also been achieved and the resulting compound shown to be biologically more potent than the parent *trans*-lactone, β-cyclocostunolide (**20**).

Several highly oxygenated melampolides which have high physiological activity have been isolated from plants of the family Compositae [3]. Our recent success in relating structure with physiological activity of terpenoids in general and terpenoid lactones in particular led us to transform some of the germacrane-type sesquiterpene lactones into melampolides via regio- and stereo-specific allylic oxidation [4] with selenium dioxide and TBHP. The major biological parameter studied was rooting in stem cuttings of *Phaseolus aureus* L.

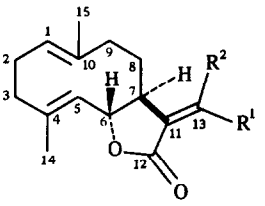
RESULTS AND DISCUSSION

Oxidation of germacranolides with SeO_2–*TBHP*

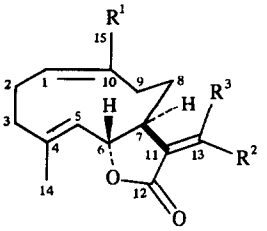
Costunolide on oxidation with SeO_2 is known to give the melampolides **4** and **5** [5]. However, in the light of a recent report [4], the oxidation was carried out with SeO_2 in combination with TBHP. The reaction occurred during a period of 2 min as compared with the reaction time of several hours reported in ref. [5]. Chromatographic separation of the mixture led to the isolation of an aldehydolactone (**4**) and a hydroxylactone (**5**), which were identical in all respects to compounds reported in the literature [5]. The presence of a melampolide system in the products of such an oxidation of compounds **2**, **3** and **13**, respectively, was proven in two ways. Firstly, the aldehydic chemical shift of the germacranolide derivatives (**7**, **10** and **14**) near δ9.5 was in good agreement with the values reported for the aldehyde protons of related melampolides [6]. Secondly, compound **5** on reaction with diazomethane afforded the corresponding pyrazoline (**17**). In this stereostructure, the –N=N– grouping was *cis* with respect to H-6 since this proton in the pyrazoline derivative ($C_{16}H_{22}O_3N_2$, mp 105°) showed an appreciable downfield shift compared with other compounds in this series (Table 1). The structure **17** was further confirmed from the IR spectrum, which showed the absence of a band at 820 cm^{-1} typical of an α-methylene in conjugation with a lactone carbonyl. The ^1H NMR spectral features (Table 1), particularly the absence of two doublets around δ6 due to two conjugated C-13 methylenic protons, were in complete accord with the stereostructure **17**. The pyrazoline on pyrolysis afforded a two-component mixture, which on chromatographic separation gave two crystalline compounds, mp 110 and 112°. These were characterized by the spectral data as **8** and **15**, respectively. The hydroxylactones **8** and **15** were identical in all respects to the hydroxylactones obtained by SeO_2–TBHP oxidation of germacranolides **2** and **13**.

Isomerization of **2** with diethylamine afforded the corresponding (*E*)-isomer (**3**), $C_{16}H_{22}O_2$, mp 58°, and this stereostructure was in complete agreement with its ^1H NMR spectrum (Table 1), which had the H-13 olefinic signal at δ6.89 compared with δ6.07 in **2**. Oxidation of **3** with SeO_2–TBHP afforded **10** and **11**. The ^1H NMR spectral features (Table 1) were in complete agreement with their structures. The hydroxylactones **5**, **8**, **11** and **15** afforded their corresponding acetates on acetylation with acetic anhydride–pyridine and all of them gave their parent aldehydes (**4**, **7**, **10**, **14**) on oxidation with active manganese dioxide.

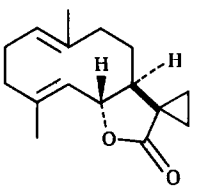
The aldehydolactones **4**, **7** and **14** were tested as root-inducing agents on hypocotyl cuttings of *P. aureus* (Table 2). Significantly these compounds were distinctly several times more active in causing rooting than their



	R^1	R^2
1	H	H
2	Me	H
3	H	Me



	R^1	R^2	R^3
4	CHO	H	H
5	CH_2OH	H	H
6	CH_2OAc	H	H
7	CHO	Me	H
8	CH_2OH	Me	H
9	CH_2OAc	Me	H
10	CHO	H	Me
11	CH_2OH	H	Me
12	CH_2OAc	H	Me



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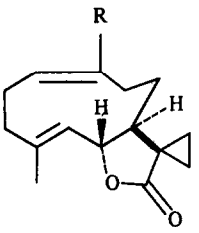
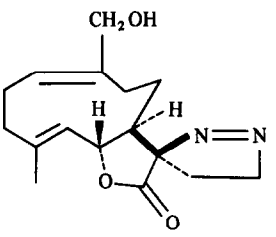


	R
14	CHO
15	CH_2OH
16	CH_2OAc



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parent compounds **1**, **2** and **13**, respectively. Thus, the aldehydolactone **4** at concentrations above 5 ppm was more than three times more active than costunolide [7]. The aldehydolactone **14** was, however, the most potent compound in this series and represents one of the most potent root initiators among the terpenoids screened in our laboratory so far. The data (Table 2) show that the aldehydolactone **14** at 15 ppm was eight times more active than costunolide (**1**) and four times more active than its parent C-16 germacranolide (**13**). This enhancement of the biological activity was probably due to the presence of two α,β-unsaturated carbonyl moieties in **4**, **7** and **14** compared to only one such moiety present in their parent compounds **1**, **2** and **13**.

Cyclization of germacranolides with pyridinium chlorochromate

In our attempts to cyclize the 1,5-cyclodecadiene system present in costunolide to obtain biogenetically feasible natural products, two reagents, i.e. pyridinium chlorochromate and iodine, were employed. The reaction of costunolide (**1**) with pyridinium chlorochromate led to the formation of a mixture of products, which on chromatographic separation afforded four crystalline compounds, mp 82°, 72°, 144° and 75°. The first two compounds were identified as α- and β-cyclocostunolides, respectively, from their mps and comparison of their IR spectra with authentic samples. The major component of the reaction

Table 1. IR (Nujol) and ^{1}H NMR ($CDCl_3$, TMS as internal standard) data of compounds **3**, **6–12** and **14–17**

Compound	IR (cm^{-1})	H-1	H-14	H-5	H-6	H-13	H-15	H-16/OAc
3	1770, 1650, 830	5.0 (1H, *m*)	1.75 (3H, *br s*)	4.85 (1H, *d*, $J=9$*)	4.50 (1H, *t*, $J=9$)	6.89 (1H, *dq*, $J=3, 8$)	1.55 (3H, *br s*)	2.0 (3H, *dd*, $J=3, 8$)
6	1735, 1760, 1650, 1210	5.50 (1H, *m*)	1.8 (3H, *br s*)	5.02 (1H, *d*, $J=9$)	4.45 (1H, *t*, $J=9$)	5.37, 6.12 (1H each, *d*, $J=3$)	ABq A=4.35, B=4.55 (2H, $J=12$)	2.0 (3H, *s*)
7	1760, 1670	6.5 (1H, *m*)	2.00 (3H, *s*)	5.15 (1H, *d*, $J=10$	4.50 (1H, *t*, $J=9$)	6.20 (1H, *dq*, $J=3, 8$)	9.55 (1H, *s*)	2.25 (3H, *dd*, $J=3, 8$)
8	3450, 1765	5.5 (1H, *m*)	1.99 (3H, *br s*)	5.15 (1H, *d*, $J=9$)	4.50 (1H, *t*, $J=9$)	6.10 (1H, *dq*, $J=3, 8$)	4.12 (2H, *s*)	2.23 (3H, *dd*, $J=3, 8$)
9	1730, 1765, 1645, 1200	5.45 (1H, *m*)	1.81 (3H, *br s*)	5.0 (1H, *d*, $J=10$)	4.50 (1H, *t*, $J=9$)	6.0 (1H, *dq*, $J=3, 8$)	ABq A=4.4, B=4.6 (2H, $J=12$)	2.18 (3H, *dd*, $J=3, 8$) 2.03 (3H, *s*)
10	1765, 1675	6.50 (1H, *m*)	1.90 (3H, *br s*)	5.05 (1H, *d*, $J=10$)	4.50 (1H, *t*, $J=9$)	6.89 (1H, *dq*, $J=3, 8$)	9.52 (1H, *s*)	2.25 (3H, *dd*, $J=3, 8$)
11	3450, 1760	5.50 (1H, *m*)	1.85 (3H, *br s*)	5.10 (1H, *d*, $J=9$)	4.49 (1H, *t*, $J=9$)	6.82 (1H, *dq*, $J=3, 8$)	4.10 (2H, *s*)	2.0 (3H, *dd*, $J=3, 8$)
12	1725, 1765	5.70 (1H, *m*)	2.0 (3H, *br s*)	5.10 (1H, *d*, $J=10$)	4.55 (1H, *t*, $J=9$)	7.00 (1H, *dq*, $J=3, 8$)	ABq A=4.4, B=4.7 (2H, $J=12$)	2.15 (3H, *s*) 2.10 (3H, *dd*, $J=3, 8$)
14	1760, 1665, 1640	6.10 (1H, *m*)	1.95 (3H, *br s*)	4.85 (1H, *d*, $J=9$)	4.40 (1H, *t*, $J=9$)	0.5–1.2 (*m*)	9.75 (1H, *s*)	0.5–1.2 (*m*)
15	3460, 1760, 1660	5.32 (1H, *m*)	1.87 (3H, *br s*)	5.0 (1H, *d*, $J=9$)	4.60 (1H, *t*, $J=9$)	0.6–1.1 (*m*)	3.85 (2H, *s*)	0.6–1.1 (*m*)
16	1760, 1740, 1660, 1210	5.45 (1H, *m*)	1.80 (3H, *br s*)	5.0 (1H, *d*, $J=10$)	4.50 (1H, *t*, $J=9$)	0.5–1.2 (*m*)	ABq A=4.2, B=4.6 (2H, $J=12$)	2.0 (3H, *s*) 0.5–1.2 (*m*)
17	3540, 1750, 1660, 830	5.45 (1H, *m*)	1.92 (3H, *br s*)	5.02 (1H, *d*, $J=9$)	5.60 (1H, *t*, $J=9$)	—	4.0 (2H, *s*)	4.7 (2H, *t*, $J=9$)

*Coupling constants (J) in Hz.

Table 2. Effect of 5, 10, 15 and 20 mg/l. germacranolide or eudesmanolide on the number of roots per rooted segment produced by hypocotyl cuttings of *P. aureus* after 7 days*

Compound	No. of roots			
	5	10	15	20
2 (*Z*)	5.1±1.1	12.5±2.7	9.8±0.7	11.8±1.5
3 (*E*)	6.7±1.1	15.0±2.2	19.3±3.7	20.0±1.6
4	10.0±1.0	16.0±2.2	17.3±2.8	Toxic
5	7.5±1.8	11.5±2.0	15.3±2.3	Toxic
6	7.1±1.5	9.0±1.7	8.3±1.2	9.5±2.3
7	15.6±2.8	17.8±2.8	20.8±2.6	18.8±1.3
8	7.2±1.2	11.4±2.8	14.6±3.2	18.0±3.3
9	9.6±1.4	13.7±2.0	14.2±1.7	21.2±4.1
14	26.8±3.8	34.3±5.1	47.3±5.8	28.7±4.6
15	5.8±1.2	10.7±1.6	11.4±1.6	14.0±1.1
16	9.0±1.3	11.0±1.1	10.0±1.2	14.8±3.3
20	7.1±0.9	18.5±1.2	16.9±1.2	Toxic
31	16.2±1.3	22.0±2.1	22.3±2.0	24.6±1.8

*Control (water) = 6.±1.9.

mixture was a hydroxylactone, $C_{15}H_{22}O_3$, mp 75°. Its NMR spectrum showed a pair of doublets at δ6.2 and 5.5 due to H_2-13, a broad singlet at δ3.1 due to –CHOH and two sharp methyl singlets at δ1.01 and 1.40. The absence of a signal due to a proton on the carbinol carbon showed that the methyl singlet at δ1.40 was due to the methyl attached to a carbon bearing the hydroxyl group. These data were indicative of a eudesmane-type skeleton in the compound. Reference to the literature [8], showed complete agreement of its mp and spectral data with those of arbusculin-A, a naturally occurring compound. The compound of mp 144° displayed IR bands at 1702 and 1760 cm^{-1} in addition to the characteristic bands for an isolated methylenic double bond in conjugation with the lactonic carbonyl. The band at 1702 cm^{-1} was suggestive of a cyclohexanone in which the α-carbon was highly substituted [9], while the band at 1760 cm^{-1} showed the presence of a γ-lactone moiety. The ^{1}H NMR spectrum displayed a pair of low-field doublets at δ6.18 and 5.47 (J = 3 Hz), corresponding to the protons of an exocyclic methylene group conjugated to a lactone carbonyl function. A methyl singlet at δ1.16 was attributed to the presence of an angular methyl group. A triplet at δ4.15 (J = 9 Hz) was assigned to a hydrogen on the lactone which had two protons on a neighbouring carbon, as shown by the diaxial coupling observed. These data also suggested the eudesmane skeleton for this compound and pointed to structure **23**. Yoshioka *et al.* [10] showed that **23** was the oxidation product of reynosin. The mps and the NMR spectral features of the compounds were in excellent agreement.

A similar reaction on **3** and **13** afforded the corresponding cyclized and oxygenated compounds, to which structures **19**, **21**, **24** and **33**, and **25**, **26**, **27** and **34** have been assigned on the basis of the spectral data (Table 3).

Pyridinium chlorochromate therefore represents an excellent reagent to bring about cyclization of the 1,5-cyclodecadiene system of a germacranolide by introducing oxygen-containing groups at C-1 and C-4 in the resulting eudesmanolides.

Cyclization of costunolides with iodine

Iodine is used to bring about double-bond isomerization [11, 12]. When a solution of costunolide in benzene was reacted with a trace of metallic iodine, a spontaneous reaction occurred affording products of cyclization. Chromatographic separation gave four compounds. Of these, α-cyclocostunolide (**18**) and β-cyclocostunolide (**20**) were identified by mp and mmp determinations with authentic samples. Of the remaining two compounds, the compound of mp 128° was an iodo derivative, $C_{15}H_{19}O_2I$, as revealed by qualitative tests and by mass spectroscopy, $[M]^+$ m/z 357; $[M-I]^+$ m/z 231. The IR spectrum showed bands at 1770 (γ-lactone), 1650, 1000 ($>C=CH_2$) and at 820 cm^{-1} (conjugated $>C=CH_2$). The ^{1}H NMR spectral features (Table 3) confirmed the presence of these groups and considering the mode of cyclization in costunolide, structure **22** was assigned to this compound. The β-configuration of the I atom at C-1 in the iodolactone (**22**) was revealed by its NMR spectrum, which had a double-doublet at δ4.3 (J = 4, 9 Hz).

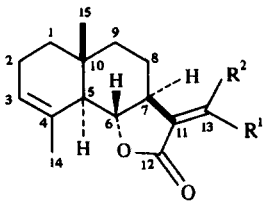
Conversion of β-cyclocostunolide to (−)-β-frullanolide

β-Cyclocostunolide (**20**) on reaction with antimony

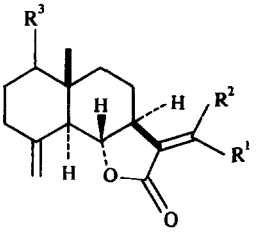
Table 3. IR (Nujol) and ^{1}H NMR ($CDCl_3$, TMS as internal standard) data of compounds **22**, **24**, **27**, **33** and **34**

Compound	IR (cm^{-1})	H-1	H-14	H-6	H-15	H-13	H-16
22	1765, 1640, 892	4.3 (1H, *dd*, J=4, 9*)	4.8, 4.9 (1H each, *s*)	3.80 (1H, *t*, J=9)	0.9 (3H, *s*)	5.35, 6.05 (1H each, *d*, J=3)	—
27	1765, 1705, 895	—	5.25, 5.30 (1H each, *s*)	4.32 (1H, *t*, J=9)	1.20 (3H, *s*)	0.6–1.0 (*m*)	0.6–1.0 (*m*)
24	1765, 1703, 1650, 900	—	5.23, 5.35 (1H each, *s*)	4.18 (1H, *t*, J=9)	1.22 (3H, *s*)	6.20 (1H, *dq*, J=3, 8)	2.25 (3H, *dd*, J=3, 8)
33	3580, 1765	—	1.30 (3H, *s*)	4.01 (1H, *t*, J=10)	0.88 (3H, *s*)	6.01 (1H, *dq*, J=3, 8)	2.15 (3H, *dd*, J=3, 8)
34	3580, 1765	—	1.28 (3H, *s*)	4.1 (1H, *t*, J=10)	0.87 (3H, *s*)	0.6–1.1 (*m*)	0.6–1.1 (*m*)

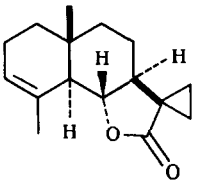
*Coupling constants (J) in Hz.



18 $R^1 = R^2 = H$
19 $R^1 = Me$, $R^2 = H$



	R^1	R^2	R^3
20	H	H	H
21	Me	H	H
22	H	H	I
23	H	H	=O
24	Me	H	=O



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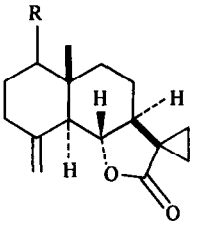
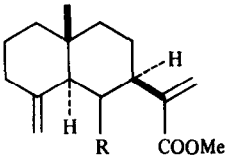
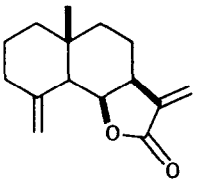


26 R, H
27 R, =O



R
28 ----- OH
29 =O
30 ▬ OH



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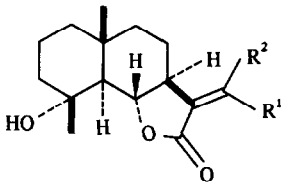
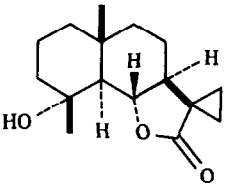


32 $R^1 = R^2 = H$
33 $R^1 = Me$, $R^2 = H$



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trichloride–methanol afforded compound **28**, which on oxidation afforded the keto-ester **29**. The keto ester on reduction with sodium borohydride followed by chromatography afforded the hydroxyester **30**. The *β*-configuration of the hydroxyl group at C-6 was revealed by a sharp singlet at $\delta 4.1$ ($W_{1/2} = 6$ Hz). The hydroxyester **30** on reaction with antimony trichloride–methanol underwent a transesterification [13] to afford the *cis*-lactone **31**, identical in all respects to (−)-*β*-frullanolide synthesized earlier [2] following a different route.

(−)-*β*-Frullanolide (**31**) was a better root promotor than its parent *β*-cyclocostunolide (**20**) (Table 3). The better biological activity of compound **31** over **20** was probably due to epimerization, a stereochemical change which is known to effect activity towards root initiation [16].

Summary of biological activity

It was known [13] that the C-16 isomer (**13**) of costunolide was a more potent root promotor than the parent compound. We have now shown that the (*E*)-isomer **3** is more active than the corresponding (*Z*)-isomer **2** (Table 2). This type of distinct change in the biological activity on *E–Z* isomerization [14] is well established and no exception has been found so far.

The biological activities of several melampolides synthesized by us are reported. The aldehydolactones **4**, **7**, **10** and **14** are more potent initiators of root formation than their parent germacranolides, **1**, **2**, **3** and **13**, respectively. The probable reasons for this are that these compounds have a melampolide skeleton and in addition to the conjugated lactone moieties [15], already established by us to enhance root formation, they contain an α,β-unsaturated aldehyde function.

It has been reported [7] that the presence of a cyclopropane ring in the α,β-position of a terpenoid γ-lactone moiety always enhances the biological activity, associated with the parent α-methylene-γ-lactone. And it is significant that **14** is a highly potent compound. The melampolides **5**, **8**, **11** and **15**, which have a primary hydroxyl group, show a diminished activity compared with their corresponding aldehydes, and an activity pattern similar to that given by the parent alcohols is observed with their esters **6**, **9**, **12** and **16**. (−)-β-Frullanolide (**31**) is more active than its parent β-cyclocostunolide (**20**). This enhanced activity of the *cis*-lactone (**31**) is reminiscent of the increase in biological activity on epimerization [16].

EXPERIMENTAL

General procedures. 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 (Na_2SO_4). After removal of the solvent, the reaction products were separated by CC on silica gel. IR: Nujol; ^{1}H NMR: $CDCl_3$, TMS as internal standard

Oxidation with SeO_2–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 5 min at room temp., the reaction was complete and afforded a mixture of products

Acetylation. The compound (0.2 g) was dissolved in a mixture of pyridine (2 ml) and Ac_2O (0.2 ml). After 12 hr at room temp., the acetylation was complete in each case.

Cyclization of eudesmanolides with pyridinium chlorochromate. Pyridinium chlorochromate was prepared as follows:

To 90 ml 6 M HCl (1.1 mol) was added 50 g (1 mol) CrO_3 rapidly with stirring. After 5 min, the homogeneous soln was cooled to 0° and 40 g (1 mol) of pyridine was carefully added over 10 min. Recooling to 0° gave a yellow-orange solid which was collected over a sintered glass funnel

To a suspension of pyridinium chlorochromate (2 g) in CH_2Cl_2 (20 ml) was added the soln of compound (2 g) in CH_2Cl_2 (5 ml). The mixture was stirred at room temp. for 12 hr. It was then diluted with Et_2O (100 ml) and the solvent decanted. Evapn of the solvent afforded a mixture of products.

Reaction of costunolide (**1**) *with SeO_2–TBHP.* Costunolide (1.0 g) was reacted with SeO_2–TBHP, and the reaction mixture chromatographed to yield **4** (0.3 g), mp 130°, as a minor component, in the petrol–Et_2O (17:3) fraction. (Found: C, 73.04; H, 7.57. $C_{15}H_{18}O_3$ requires: C, 73.14; H, 7.37%.) Further elution with petrol–Et_2O (20:7) yielded **5** (0.6 g), mp 95° as the major component. (Found: C, 72.46; H, 8.22. $C_{15}H_{20}O_3$ requires: C, 72.55; H, 8.12%.)

Reaction of **2** *with SeO_2–TBHP.* Compound **2** (1.5 g) was treated with SeO_2–TBHP and the reaction mixture chromatographed to afford **7** (0.4 g), mp 107°, with petrol–Et_2O (4:1). (Found: C, 73.80; H, 7.84. $C_{16}H_{20}O_3$ requires: C, 73.82, H, 7.74%.) Further elution with petrol–Et_2O (2:1) afforded **8** (1.0 g), mp 112°, as a major component (Found: C, 73.35; H, 8.30. $C_{16}H_{22}O_3$ requires: C, 73.25; H, 8.45%.)

Reaction of **13** *with SeO_2–TBHP.* Compound **13** (1 g) was treated with SeO_2–TBHP and the reaction mixture chromatographed to furnish **14** (0.3 g), mp 155°, on elution with petrol–Et_2O (17:3). (Found: C, 73.80; H, 7.82. $C_{16}H_{20}O_3$ requires: C, 73.82; H, 7.74%.) Further elution of the column with petrol–Et_2O (5:3) afforded **15** (0.6 g), mp 112°. (Found. C, 73.35, H, 8.31. $C_{16}H_{22}O_3$ requires: C, 73.25; H, 8.45%.)

Pyrazoline (**17**) A soln of **5** in Et_2O on reaction with excess CH_2N_2 gave pyrazoline (**17**), mp 105°, in quantitative yield. (Found: C, 66.17; H, 7.63. $C_{16}H_{22}O_3N_2$ requires: C, 66.18; H, 7.64%.) Pyrolysis of **17** (2 g) at 120° for 1 hr afforded a two-component mixture, which on chromatography yielded **8** (0.7 g), mp 110°, with petrol–Et_2O (5:2). Further elution of the column with petrol–Et_2O (2:1) yielded **15** (1.2 g), mp 112°. These compounds were found to be identical to the compounds obtained by SeO_2–TBHP oxidation of **2** and **13**.

MnO_2 *oxidation of compounds* **5**, **8**, **11** *and* **15**. These compounds on oxidation with active MnO_2 in $CHCl_3$ gave **4**, **7**, **10** and **14** in quantitative yields.

Acetylation of **5**, **8**, **11** *and* **15**. Acetylation of **5** afforded a crystalline compound **6**, mp 89°. (Found: C, 70.31; H, 7.62; $C_{17}H_{22}O_4$ requires: C, 70.32; H, 7.64%.) Similarly, acetylation of **8**, **11** and **15** yielded compounds **9**, mp 150° (Found: C, 71.01; H, 7.96. $C_{18}H_{24}O_4$ requires: 71.02; H, 7.95%); **12**, mp 61° (Found: C, 71.02; H, 7.95. $C_{18}H_{24}O_4$ requires: C, 71.01; H, 7.92%); and **16**, mp 78° (Found: C, 71.00; H, 7.93. $C_{18}H_{24}O_4$ requires: C, 71.06, H, 7.91%).

Isomerization of **2**. Compound **2** (1 g) in EtOH (150 ml) was stirred with Et_2NH (5 ml) at 50° for 12 hr. The products were chromatographed to furnish **2**, upon elution with petrol–Et_2O (20:1). Further elution with petrol–Et_2O (10:1) afforded its isomer **3** (0.5 g), mp 58°. (Found: C, 78.11; H, 8.99. $C_{16}H_{22}O_2$ requires: C, 78.01; H, 9.00%.)

Reaction of costunolide (**1**) *with pyridinium chlorochromate.* Compound **1** (3 g) was treated with pyridinium chlorochromate in CH_2Cl_2 and the reaction mixture chromatographed to give **18** (*ca* 1 g), mp 82°, with petrol–Et_2O (33:1) and **20**, mp 72°, on elution with the same solvent in traces. Further elution of the column with petrol–Et_2O (10:1) gave compound **23** (1.2 g), mp 144° (Found: C, 73.06; H, 7.53. $C_{15}H_{18}O_3$ requires: C, 73.14; H, 7.37%), and finally elution with petrol–Et_2O (20:3) gave **32** (1.5 g), mp 75° (Found: C, 71.88; H, 8.78. $C_{15}H_{22}O_3$ requires: C, 71.97; H, 8.86%).

Reaction of **2** *with pyridinium chlorochromate.* Compound **2** (3 g) was treated with pyridinium chlorochromate to furnish a mixture of products, which on chromatographic separation gave compound **19** (0.3 g), a yellow liquid, with petrol–Et_2O (33:1) and **21** (0.2 g), mp 88° with the same solvent system. Further elution of the column with petrol–Et_2O (20:1) gave compound **24** (1.2 g), mp 131° (Found: C, 73.81; H, 7.82. $C_{16}H_{20}O_3$ requires: C, 73.82; H, 7.74%), and on further elution with petrol–Et_2O (10:1) compound **33** (1.4 g), mp 72° (Found: C, 72.68; H, 9.05. $C_{16}H_{24}O_3$ requires: C, 72.69; H, 9.15%), was obtained.

Reaction of compound **13** *with pyridinium chlorochromate.* Compound **13** (3 g) was treated with pyridinium chlorochromate to give a mixture of products, which was subjected to chromato-

graphic separation. Elution of the column with petrol–Et_2O (33:1) gave **25** (0.2 g), mp 73°, and **26** (0.3 g), mp 79°, with petrol–Et_2O (33:1). Further elution with petrol–Et_2O (20:1) gave **27** (0.75 g), mp 120° (Found: C, 73.80; H, 7.81. $C_{16}H_{20}O_3$ requires: C, 73.82; H, 7.74%), and on further elution with petrol–Et_2O (20:3) **34** (1.2 g), mp 111° (Found: C, 72.61; H, 9.01. $C_{16}H_{24}O_3$, requires: C, 72.69; H, 9.15%) was obtained.

Cyclization of costunolide (**1**) *with* I_2. To a soln of costunolide (1 g) in dry C_6H_6 (100 ml) was added a soln of I_2 in dry C_6H_6 (10 ml). The mixture was warmed at 40° for 1 min when all of the costunolide reacted. The mixture was washed with aq. $Na_2S_2O_3$ and then chromatographed. Elution of the column with petrol–Et_2O (33:1) afforded 0.2 g α-cyclocostunolide (**18**), mp 82°, mmp with an authentic sample undepressed. Further elution with the same solvent furnished β-cyclocostunolide (0.2 g), mp 72°, mmp with an authentic sample undepressed. Elution of the column with petrol–Et_2O (20:1) afforded **22** (0.3 g), mp 128°, $C_{15}H_{19}O_2I$, $[M]^+$ *m/z* 357, $[M-I]^+$ *m/z* 251. Finally elution with Et_2O afforded a compound (0.2 g), mp 158°.

Hydroxyester **30**. Reaction of β-cyclocostunolide (**20**, 2 g), with $SbCl_3$–MeOH afforded **28** (0.8 g), which on oxidation with Jones reagent afforded **29** (0.75 g), $C_{16}H_{22}O_3$.

A soln of **29** (1 g) in MeOH (5 ml) was reduced with a soln of $NaBH_4$ (0.4 g) in MeOH (5 ml). After 12 hr at room temp., the product was isolated in the usual way and chromatographed. Elution of the column with petrol–Et_2O (20:1) furnished **30** (0.7 g), mp 66° (Found: C, 72.61; H, 9.09. $C_{16}H_{24}O_3$ requires. C, 72.69; H, 9.15%).

(−)-*β-Frullanolide*. Compound **30** (0.5 g) in MeOH (5 ml) was treated with $SbCl_3$ (2.0 g) in MeOH (5 ml). The reaction mixture after 0.5 hr on a steam bath was poured into H_2O and extracted with Et_2O to afford a crystalline solid, **31** (0.4 g), mp 153°; IR spectrum was identical to that of an authentic sample of (−)-β-frullanolide

Biological testing. P. 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, four concentrations (5, 10, 15 and 20 mg/l.) together with H_2O as control were tested. For all treatments, 10 replicates were cultured in vials, each containing 30 ml test soln. The final observations were made on day 8. The experiment was repeated (3 ×) at 27 ± 2°.

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