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PLANT GROWTH ACTIVITY OF GUAIANOLIDES WITH C-4 OXYGEN-CONTAINING GROUPS

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Key Word Index—*Phaseolus aureus*; Leguminosae; guaianolides; dehydrocostus lactone; plant growth activity; α-methylene-γ-lactone.

Abstract—The plant growth activity of guaianolides with C-4 oxygen-containing functions has been studied. It was found that the presence of an epoxy or an ether link at C-4 enhanced the plant growth activity of the parent compound. The presence of a hydroxyl group at C-4 had no effect.

INTRODUCTION

We have shown that the root-promoting activity of dehydrocostus lactone (**1**) is enhanced [1] by the introduction of an epoxy group at C-4 (**2**). We, therefore, decided to introduce different oxygen functions (hydroxy, ether, epoxy) at this position in different conjugated γ-lactones to study their plant growth activity. The present paper reports the preparation of eight hitherto unknown compounds and analyses the structure–reactivity relationship in the case of guaianolides. The present work is restricted to guaianolides since it has been observed that a large variation in biological activity is displayed by compounds having similar active moieties but different underlying carbon skeleton (P. S. Kalsi, unpublished work).

RESULTS AND DISCUSSION

Epoxidation studies

Epoxidation of **6** with perbenzoic acid afforded, after chromatography, **8** (mp 92°) and **7** (mp 76°). The IR and ^{1}H NMR spectra of these compounds showed the presence of an olefinic methyl, and an olefinic proton which was part of a conjugated lactone system. The signal for the C-6 proton was unaltered, which showed that there had been no change in stereochemistry at C-5, C-6 and C-7. Both compounds also contained one exomethylenic function and the two mutually coupled protons of an oxirane ring. The compound with the higher mp was assigned structure **8**, as the exocyclic methylene protons were well separated (δ 4.95, 5.25); cf. the ^{1}H NMR spectra of the compound with the lower mp in which the exocyclic methylene proton appeared as a singlet as it was not influenced by the ether oxygen function at C-6. We could not assign the stereochemistry of the epoxide ring of **8**, whereas for **7** we placed the epoxy ring in the β-position as the oxirane protons were separated by δ 0.5. Inspection of a model shows that with an α-epoxide these two hydrogens would not be well separated.

Epoxidation of **10** afforded only one epoxide (mp 115°). Based on the spectral features and arguments similar to those used above, it was possible not only to place the epoxy ring at C-4 but also to fix the stereochemistry as in **12**.

Oxymercuration–demercuration

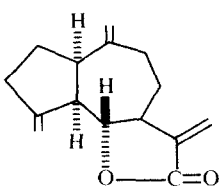
To introduce an ether or hydroxyl function at C-4, oxymercuration–demercuration was carried out. Govindan and Bhattacharyya [2] reported that with dehydrocostus lactone (**1**) this reaction gave an ether (**13**) and a hydroxy compound (**5**). In our hands, however, **1** gave an extra alcoholic compound which was assigned structure **4** on the basis of its ^{1}H NMR spectra. Oxymercuration–demercuration of **6** led to the isolation of two compounds, mp 118° and 136°, the spectral data of which were consistent with them having structures **14** and **9**, respectively. A similar reaction on compound **10** afforded two crystalline compounds, **15** (mp 144°) and **11** (mp 178°).

Stereochemical aspects

In the ^{1}H NMR spectra of the ethers **13**, **14** and **15** the C-6 protons appeared as doublets cf. the triplets seen in the other compounds of the series. This was due to a distortion of the dihedral angle between the C-5 and C-6 protons to approximately 100°, thus reducing the coupling constant to a very small value. The mode of formation, models and NMR data fixed the stereochemistry of the ether bridge as β, a conclusion also reached by other workers [2] for **3**. The C-6 proton in compounds **5** and **11** was considerably deshielded from its normal position of approximately δ 4. This deshielding was consistent with a β-placement of the C-10 hydroxyl in these compounds. The stereochemical assignments of **7**, **9**, **11** and **12** were based only on NMR data and should, therefore, be treated as tentative.

Biological activity

A recent review [3] records some of the structural features required for biological activity of sesquiterpene lactones. These features include the presence of an exomethylene group conjugated with the γ-lactone. Further, it has been reported that the presence of a



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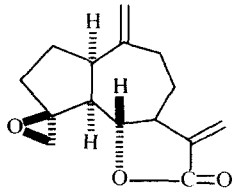

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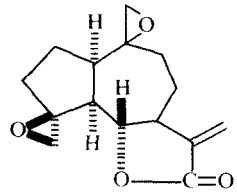

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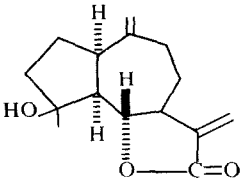

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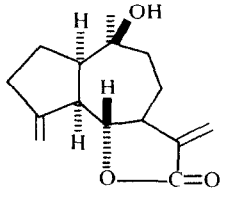

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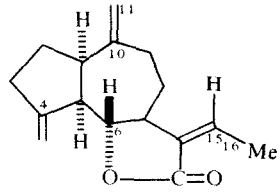

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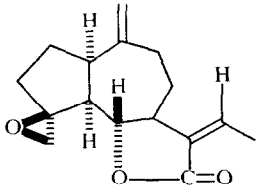

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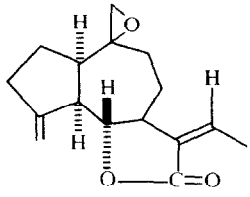

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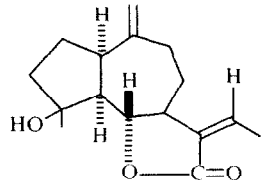

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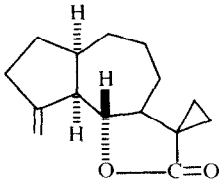

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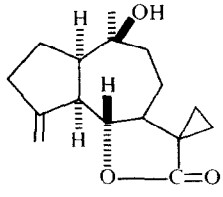

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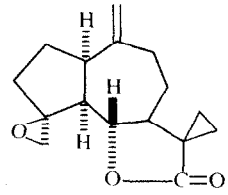

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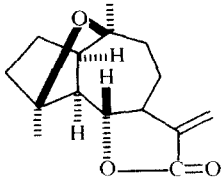

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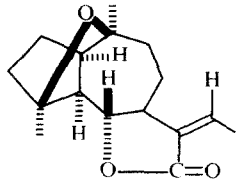

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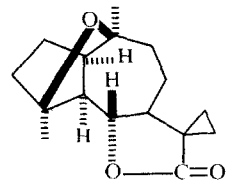

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functional group, such as an epoxide, a hydroxyl, a chlorohydrin, an unsaturated ketone or an O-acyl adjacent to the α-methylene of the γ-lactone, enhances the activity of the conjugated lactone towards biological nucleophiles, thus increasing their bioactivity. We had already established [1] that the introduction of an epoxide group at C-4, as in **2** and **3**, enhanced the root-forming potential of the parent α-methylene-γ-lactone moiety of dehydrocostus lactone (**1**). This observation now receives further support since it was found that **7** and **12** were more potent root-forming agents than their parent lactones **6** and **10** (Table 1). The new data also showed that **8**, in which the epoxide group is placed at a remote position from the α-methylene-γ-lactone moiety, did not bring about the rooting enhancement shown by **7**. The introduction of a second epoxy group at a remote position did not add further to the enhancement of rooting potential produced by the first epoxy group at C-4 since, as shown previously, **3** was almost as active as **7** [1]. The introduction of a hydroxyl group at C-4 instead of an

Table 1. Effect of different concentrations of terpenoids on the number of roots per rooted segment produced on hypocotyl cuttings of *P. aureus* after 7 days

Compound	Number of roots			
	10*	20*	30*	40*
1	8.4 ± 1.01	9.6 ± 1.01	11.0 ± 1.41	—
4	10.0 ± 1.41	11.2 ± 2.35	7.5 ± 0.57	11.3 ± 1.35
5	10.3 ± 0.46	8.0 ± 0	9.3 ± 1.24	15.3 ± 2.02
6	10.0 ± 2.34	17.0 ± 2.44	17.3 ± 1.81	22.0 ± 2.44
7	11.6 ± 2.33	25.5 ± 3.35	26.5 ± 2.06	33.1 ± 2.42
8	5.0 ± 0.63	5.2 ± 0.74	12.6 ± 1.01	14.5 ± 1.11
9	6.2 ± 1.60	9.0 ± 0	9.5 ± 1.50	—
10	7.6 ± 0.80	20.2 ± 1.67	20.6 ± 2.33	7.5 ± 0.50
11	9.3 ± 0.83	10.0 ± 2.12	21.8 ± 2.69	—
12	8.8 ± 0.83	8.8 ± 0.83	17.0 ± 0.63	18.4 ± 0.41
13	11.0 ± 1.70	16.5 ± 1.65	22.3 ± 2.49	12.7 ± 0.46
14	16.0 ± 0.81	27.7 ± 2.16	47.7 ± 2.77	—
15	15.7 ± 2.27	30.0 ± 1.41	27.3 ± 3.56	22.6 ± 2.49
Water (control)	6.3 ± 0.47			
IAA (10 ppm)	14.2 ± 1.6			

* Concentration (mg/l.) of test compound.

epoxy group did not lead to enhancement of the activity (Table 1). Thus, **4** was almost as active as dehydrocostus lactone (**1**). Moreover, introduction of the hydroxyl group at a remote position as in **5** also had no effect. This observation was further supported by the finding that **11** exhibited the same activity as **10**. It is of interest that *unlike compounds* **1**, **4**, **5** *and* **10**, **11** *was much less active* than its parent lactone, **6**. Introduction of an ether bridge between C-4 and C-10 enhanced root formation tremendously and as a result **14** was almost three times as active at 30 ppm as its parent lactone, **6**. A definite increase in the activity of the parent compounds **1** and **10** was again observed with their corresponding ethers **13** and **15**, although to a lesser degree.

In conclusion it may be stated that the introduction of either an epoxy group at C-4 or an ether linkage between C-4 and C-10 increases the plant growth activity of the parent conjugated γ-lactone moieties in the case of guaianolides. Among the large number of conjugated lactones and their oxygenated derivatives screened in our laboratory, *compounds* **6** *and* **14** *are the most potent root* promoters.

EXPERIMENTAL

Mps are uncorr. Si gel was used for column chromatography and TLC. ^{1}H NMR (60 MHz) spectra were measured in CCl_4 with TMS as internal standard. The biological activity data are presented in Table 1.

Reaction of **6** *with perbenzoic acid.* A soln of **6** (mp 74°, 1.65 g) in $CHCl_3$ (15 ml) was reacted with 1 mol of perbenzoic acid (3b ml, 0.35 N perbenzoic acid). After 20 hr at 0° the reaction mixture was washed with aq. $NaHCO_3$, neutralized, dried (Na_2SO_4) and evapd to afford a three-component mixture which was subjected to column chromatography. Elution of the column with petrol–Et_2O (19:1) afforded the parent compound (**6**, mp 74°, mmp 74°), while the tail fractions from the same solvent systems yielded **8** (mp 92°) in 40% yield. [Found: C, 73.40; H, 7.40. $C_{16}H_{20}O_3$ requires: C, 73.85; H, 7.74%]. IR ν_{max}^{Nujol} cm^{-1}: 1750 (γ-lactone), 1650 and 875 (methylenic double bond); ^{1}H NMR: δ 3.97 (1H, *t*, *J* = 9.5 Hz C-6), 6.03 (1H, *dq*, *J* = 3 and 7 Hz, C-15), 2.17 (3H, *d*, *J* = 3 and 7 Hz, C-16-Me), 2.8 (2H, *m*, C-11), 4.97 (1H, *s(br)*, C-12), 5.25 (1H, *s(br)*, C-12). Elution of the column with petrol–Et_2O (9:1) afforded **7** (mp 76°) in 50% yield [Found: C, 73.93; H, 7.90. $C_{16}H_{20}O_3$ requires: C, 73.85; H, 7.74%]. IR ν_{max}^{Nujol} cm^{-1}: 1745, 1625, 895. ^{1}H NMR: δ 3.97 (1H, *t*, *J* = 10 Hz, C-6), 6.05 (1H, *dq*, *J* = 2 and 7 Hz, C-15), 2.1 (3H, *dd*, *J* = 3 and 7 Hz, C-16-Me), 4.91 (2H, *s(br)*, C-12), 2.85 (1H, *d*, *J* = 5 Hz, C-12), 3.35 (1H, *d*, *J* = 15 Hz, C-12).

Epoxidation of **10** with 1 mol of perbenzoic acid at 0° for 20 hr afforded only **12** (mp 115°) [Found: C, 73.50; H, 7.20. $C_{16}H_{20}O_3$ requires: C, 73.85; H, 7.74%). IR ν_{max}^{Nujol} cm^{-1}: 1760, 1640, 900. ^{1}H NMR: δ 4.15 (1H, *t*, *J* = 9.5 Hz, C-6), 0.65–1.15 (4H, *m*, C-15 and C-16-Me), 4.95 (2H, *s(br)*, C-11), 2.70(1H, *d*, *J* = 5 Hz, C-12), 3.1 (1H, *d*, *J* = 5 Hz, C-12).

Oxymercuration–demercuration of dehydrocostus lactone (**1**). Oxymercuration–demercuration was carried out as reported in ref. [2]. The viscous liquid obtained from this reaction was subjected to column chromatography. Unreacted lactone (TLC) was eluted with C_6H_{14}–EtOAc (19:1). **13** (mp 104°, lit. mp 104°) was eluted with C_6H_{14}–EtOAc (9:1) and **5** (solid; mp 117°, lit. mp 117°) was eluted with C_6H_{14}–EtOAc (5:1). The last fractions of the latter solvent contained **4** (liquid), 16% yield. (Found: C, 72.40; H, 8.02. $C_{15}H_{20}O_3$ requires: C, 72.55; H, 8.12%). IR ν_{max}^{Nujol} cm^{-1}: 3450 (—OH), 1750, 885, 830; ^{1}H NMR: δ 4.02 (1H, *dd*, *J* = 9.5 and 10.5 Hz, C-6), 5.47 (1H, *d*, *J* = 3 Hz, C-15), 6.17 (1H, *d*, *J* = 3 Hz, C-15), 4.95 (2H, *s(br)*, C-11) 1.3 (3H, *s*, C-12-Me), 6.62 (1 H, exchangeable OH).

Oxymercuration–demercuration of **6** (mp 74°) gave two compounds. Chromatographic separation with C_6H_{14} containing increasing amounts of EtOAc (5, 10 and 20%) yielded in the initial fractions the unreacted parent compound **6** (mp 74°, mmp 74°). From the middle fractions **14** (mp 118°) was obtained in 60% yield. (Found: C, 73.40; H, 8.90. $C_{16}H_{22}O_3$ requires: C, 72.25; H, 8.45%). IR ν_{max}^{Nujol} cm^{-1}: 1750, 1650; ^{1}H NMR: δ 3.9 (1H, *d(br)*, *J* = 11 Hz, C-6), 5.97 (1H, *dq*, *J* = 2 and 8 Hz, C-15),

2.11 (3H, *dd*, J = 3 and 8 Hz, C-16-Me), 1.22 or 1.28 (3H, *s*, C-11-Me or C-12-Me), 1.28 or 1.22 (3H, *s*, C-11-Me or C-12-Me). The last fractions afforded another crystalline compound, **9** (mp 136°) in 25% yield. (Found: C, 73.10; H, 8.10. $C_{16}H_{22}O_3$ requires: C, 73.25; H, 8.45%). IR ν_{max}^{Nujol} cm^{-1}: 3450 (—OH), 1745; ^1H NMR: δ 4.15 (1H, *t*, J = 9.5 Hz, C-6), 6.1 (1H, *dq*, J = 3 and 8 Hz, C-15), 2.15 (3H, *dd*, J = 3 and 8 Hz, C-16-Me), 4.9 (1H, *s(br)*, C-11), 5.07 (1H, *s(br)*, C-11), 1.5 (3H, *s*, C-12-Me).

Oxymercuration–demercuration of **10** (mp 70°) afforded a three-component mixture. Chromatographic separation with C_6H_{14}–EtOAc (9:1) yielded the unreacted parent compound **10**, (mp 70°, mmp 70°). Elution with C_6H_{14}–EtOAc (9:1) afforded **15** (mp 144°) in 60% yield. (Found: C, 73.90; H, 8.90. $C_{16}H_{22}O_3$ requires: C, 73.25; H, 8.45%). IR ν_{max}^{Nujol} cm^{-1}: 1760; ^1H NMR: δ 4.0 (1H, *d(br)*, J = 11 Hz, C-6), 0.5–1.0 (4H, *m*, C-15 and C-15-Me), 1.21 or 1.31 (3H, *s*, C-11-Me or C-12-Me), 1.31 or 1.21 (3H, *s*, C-11-Me or C-12-Me). **11** (mp 178°) was obtained in the last eluate with the same solvent in 30% yield. (Found: C, 73.63; H, 8.49. $C_{16}H_{22}O_3$ requires: C, 73.25; H, 8.45%). IR ν_{max}^{Nujol} cm^{-1}: 3450 (—OH), 1745; ^1H NMR: δ 4.65 (1H, *t*, J = 9.5 Hz, C-6), 0.6–1.2 (4H, *m*, C-15 and C-15-Me), 1.25 (3H, *s*, C-11-Me), 4.92 (1H, *s(br)*, C-12), 5.2 (1H, *s(br)*, C-12), 2.17 (1H, exchangeable OH).

Biological testing. Root initiation was measured by using hypocotyl cuttings of *Phaseolus aureus* seedlings raised under continuous illumination. After 4 days, when the hypocotyls were 4–5 cm long, cuttings were made by excision 3 cm below the cotyledonary node leaving the cotyledonary leaves and apex intact. The hypocotyls were then cultured in glass vials each containing 30 ml of test soln. The compounds were tested at four concns (10, 20, 30 and 40 mg/l.). IAA (10 ppm) was used as a standard and a H_2O control was included. Ten replicates were set up for each treatment. The solutions in the vials were replaced with fresh ones after 4 days. The final measurements were taken on the 8th day. The experiment was repeated three times at 26 ± 2°.

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REFERENCES

1. Kalsi, P. S., Kaur Parminder and Chhabra, B. R. (1979) *Phytochemistry* **18**, 1977.
2. Govindan, S. V. and Bhattacharyya, S. C. (1978) *Indian J. Chem.* **16B**, 1.
3. Rodriguez, E., Towers, G. H. N. and Mitchell, J. C. (1976) *Phytochemistry* **15**, 1973.