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STEREOSTRUCTURES OF INUNAL AND ISOALLOALANTOLACTONE, TWO BIOLOGICALLY ACTIVE SESQUITERPENE LACTONES FROM *INULA RACEMOSA*

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Key Word Index—*Inula racemosa*; Compositae; sesquiterpene lactone; eudesmanolides; alantolactones.

Abstract—Two new sesquiterpene lactones, inunal and isoalloalantolactone, have been isolated from *Inula racemosa*. Inunal, an aldehydolactone, displays considerable biological activity as a plant growth regulator. Structures have been assigned to these compounds on the basis of spectral data and chemical correlation with alantolactone and isotelekin.

INTRODUCTION

In order to learn more about the structure and biological activity relationships among the sesquiterpene lactones of *Inula racemosa* L., we initiated a systematic chemical investigation on this plant. We now report on the isolation and structure determination of two new sesquiterpene lactones which we have named inunal and isoalloalantolactone, in addition to the isolation of several known sesquiterpene lactones.

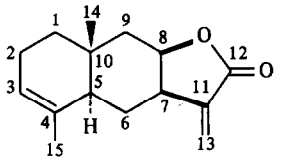
RESULTS AND DISCUSSION

The separation was followed by TLC and prep. TLC and emphasis was placed on the isolation and structure elucidation of only those compounds which were found to cause adventitious root formation [1] on stem cuttings of *Phaseolus aureus* L. This approach led to the isolation of a new aldehydolactone, which has been named inunal, and a new lactone isomeric with known alloalantolactone, which has been named isoalloalantolactone.

Isoalloalantolactone (**1**) has the composition $C_{15}H_{20}O_2$. Its IR and UV spectra showed the presence of an α-methylene-γ-lactone moiety (IR ν_{max} cm^{-1}: 1760, 1660, 815; UV λ_{max} nm (ε): 210 (15 000). Its ^1H NMR spectrum contained the characteristic pair of doublets at δ5.54 and 6.28 (1H each, *d*, $J = 3.0$ Hz) of a methylene group conjugated with a lactonic carbonyl group. Treatment of **1** with diazomethane afforded a liquid pyrazoline (**2**), the NMR spectrum of which no longer displayed the signals for the protons of the exocyclic methylene group. The signal for H-8 (Table 1) in pyrazoline (**2**) appeared at δ5.58. This shift was consistent with the relative stereochemistry at C-8 and C-11. These observations, together with the presence of a sharp three-proton singlet at δ0.95 (angular methyl), a three-proton singlet at δ1.68 (vinylic methyl) and a one proton multiplet at δ5.39 characteristic of an olefinic proton on a trisubstituted double bond indicated that isoalloalantolactone is an eudesmanolide. The position of the closure of the lactone ring was revealed by the ^1H NMR signal for the proton on the carbon atom linked to the lactonic oxygen atom. That this carbon atom is at C-8 and not at C-6 was shown by the multiplicity of the NMR signal at δ4.76, the appearance of which was consistent only with the spin coupling of a proton with three adjacent hydrogen atoms. These NMR features were very similar to those shown by alantolactones and, thus, confirmed stereostructure **1** for isoalloalantolactone. This structure was further confirmed by catalytic hydrogenation in acetic acid using platinum oxide to afford a compound with mp 142°, the spectral features (Table 1) of which were identical with those of tetrahydroalantolactone (**3**) [2].

Another compound ($C_{15}H_{22}O_2$) isolated by prep. TLC had the same ^1H NMR properties as alloalantolactone (**4**) isolated earlier [3] from this oil. It was further characterized by the crystalline pyrazoline derivative (**5**) ($C_{16}H_{22}O_2N_2$), mp 145°, obtained from the product of its reaction with diazomethane.

Inunal (**6**), $C_{15}H_{18}O_3$, mp 160°, was isolated from the more polar fractions. It displayed considerable biological activity in the adventitious root formation test and was considerably more active (Table 2) than alantolactone, isoalantolactone and isoalloalantolactone. Its IR and UV spectra showed the presence of both an α-methylene-γ-lactone and an α,β-unsaturated aldehyde function (IR ν_{max} cm^{-1}: 1770, 2725, 1680, 825; UV λ_{max} nm (ε): 236 (17, 710). Treatment of **6** with diazomethane afforded a monopyrazoline (**7**), the NMR spectrum of which contained no signals for the protons of an exocyclic methylene group. The position of the signal for the C-8 proton (δ5.58) compared to that in the parent compound was consistent with stereostructure **7**. The pyrazoline (**7**) contained an intact α,β-unsaturated aldehyde function and a pyrazoline moiety as revealed by its IR (ν_{max} cm^{-1}: 2760, 1760, 1550), UV [λ_{max} nm (ε): 237 (12 640), 325 (310)] and ^1H NMR spectra (Table 1). Its ^1H NMR spectrum showed a singlet at δ9.34 (1H) which confirmed the presence of an α,β-unsaturated aldehyde and also established the fact that there were no α-protons. The proton of the trisubstituted double bond appeared as a broad singlet at δ6.75 confirming that the trisubstituted double bond was conjugated to the aldehydic group. Two characteristic low field broadened singlets at δ5.65 and 6.10 were assigned to the protons of an exocyclic meth-



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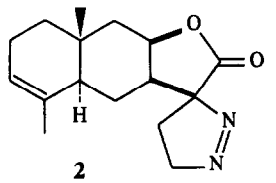

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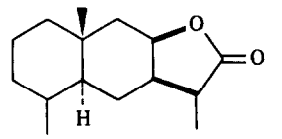

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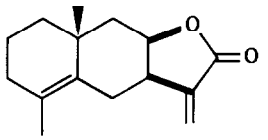

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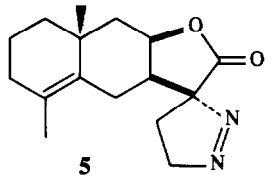

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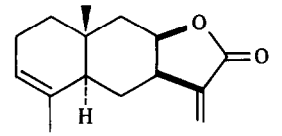

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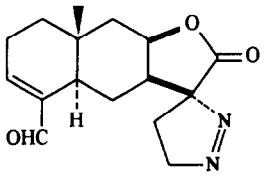

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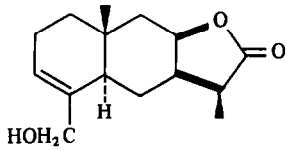

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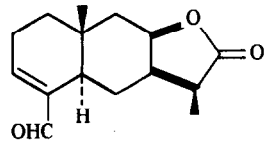

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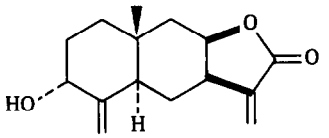

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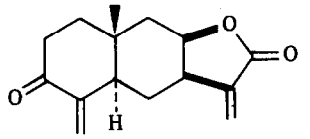

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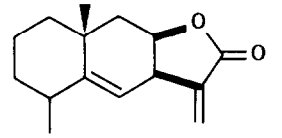

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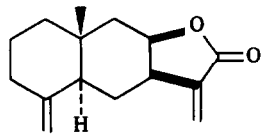

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ylene conjugated with the lactone carbonyl function. An angular methyl group at δ0.94 appeared as a broadened singlet while the sole proton on the carbon to which the ethereal oxygen of the lactone ring is attached displayed a one proton multiplet at δ4.48 typical of alantolactones. Based on this data, structure **6** appeared as a possibility.

These assignments were confirmed by reduction of **6** with sodium borohydride to afford the corresponding allylic alcohol (**8**), $C_{15}H_{22}O_3$, in which the exocyclic methylene conjugated with the lactone carbonyl was reduced. The ^{1}H NMR spectrum was in complete accord with structure **8**, in that it contained a singlet at δ0.92 (3H) for an angular methyl group, a doublet at δ1.22, (3H, J = 9 Hz) for the newly created secondary methyl group and broadened 1H singlets at δ5.76 and 4.56 for the protons on the trisubstituted double bond and on the lactone ring, respectively, while the protons of the newly created hydroxymethylene appeared as an AB quartet (δ_A 4.0 and δ_B 4.25, J = 12 Hz). On oxidation with manganese dioxide the alcohol group of **8** was oxidized back to an

Table 1. Spectral data of alantolides

Compound	IR (cm^{-1})	^1H NMR					
		H-3	H-8	H-13	H-14	H-15	H-16
1	1760, 1660, 1645, 840, 815	5.39 (1H, *m*)	4.76 (1H, *m*)	5.54, 6.28 (1H each, *br s*)	0.94 (3H, *s*)	1.68 (3H, *br s*)	—
2	1765, 1660, 1645, 1540, 1470, 845, 810	5.58 (1H, *m*)	5.58 (1H, *m*)	—	1.12 (3H, *s*)	1.66 (3H, *br s*)	4.72 (2H, *m*)
3	1765, 1385, 1340, 1300, 1175, 1090, 1030, 970, 930	—	4.26–4.42 (1H, *m*)	1.08 (3H, *d*)	1.01 (3H, *s*)	0.85 (3H, *d*)	—
5	1760, 1665, 1640, 1550, 940, 845, 815	—	5.45 (1H, *m*)	—	1.14 (3H, *s*)	1.58 (3H, *br s*)	4.62 (2H, *m*)
6	2725, 1770, 1680, 1640, 825	6.75 (1H, *br s*)	4.48 (1H, *m*)	5.65, 6.10 (1H each, *br s*)	0.94 (3H, *s*)	9.34 (1H, *s*)	—
7	2760, 1760, 1670, 1630, 1550, 1460, 1365, 875, 815	6.90 (1H, *br s*)	5.58 (1H, *m*)	—	1.0 (3H, *s*)	9.52 (1H, *s*)	4.74 (2H, *m*)
8	3580, 1770, 1670, 1160, 855	5.76 (1H, *br s*)	4.56 (1H, *m*)	1.22 (3H, *d*, $J = 9.0$ Hz)	0.92 (3H, *s*)	4.0, 4.25 (2H, AB*q*, $J = 12$ Hz)	—
9	2765, 1775, 1690, 1635, 1180, 970, 820	6.72 (1H, *br s*)	4.43 (1H, *m*)	1.15 (3H, *d*, $J = 7.0$ Hz)	0.85 (3H, *s*)	9.38 (1H, *s*)	—
11	1770, 1720, 1642, 1475, 1385, 880, 826	—	4.65 (1H, *m*)	5.72, 6.27 (1H each, *br s*)	1.0 (3H, *s*)	5.19, 5.91 (1H each, *br s*)	—

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

Compound	Number of roots* [mean ± s.e. (30)]			
	5	10	15	20
1	17.4 ± 3.6	19.4 ± 2.7	—	—
6	12.0 ± 3.5	27.7 ± 4.5	23.6 ± 3.2	27.6 ± 4.2
10	6.4 ± 0.6	8.7 ± 1.1	9.6 ± 1.6	12.4 ± 2.1
11	11.7 ± 3.8	15.0 ± 3.0	—	—
12	8.0 ± 2.5	12.2 ± 2.3	—	—
13	19.4 ± 3.6	17.3 ± 3.7	—	—

*Control experiment, water: 6.6 ± 0.9 (mean no. of roots ± s.e.).

α,β-unsaturated aldehyde. The IR, UV and ^1H NMR spectral data of the resultant product were in complete accord with structure **9** (Table 1).

Oxidative rearrangement of secondary allylic alcohols with chromium (VI) reagents to afford α,β-unsaturated carbonyl compounds have been reported [4]. These rearrangements have been found useful for effecting the 1,3-transposition of oxygen. A similar oxidative rearrangement on isotelekin of the established stereostructure **10** was, therefore, expected to afford inunal. Reaction of isotelekin (**10**), isolated from this oil, with pyridinium chlorochromate afforded a two-component mixture which was separated by chromatography to afford inunal (**6**) (mp 160°, $C_{15}H_{18}O_3$, IR and mmp) and 3-oxoisoalantolactone (**11**) (mp 145°, IR and NMR, Table 1) [5]. These data confirmed the stereostructure of inunal as shown in **6**.

From the more polar fractions of the oil which lacked biological activity, a compound, mp 144°, $C_{15}H_{20}O_3$, was isolated in pure form and shown (IR, ^1H NMR) to be identical with isotelekin (**10**) (lit. mp 144°) earlier isolated from *Telekia speciosa* [6] and *Ambrosia confertiflora* [7].

Structure–biological activity relationship

The α-methylene-γ-lactones isoalantolactone (**13**), alantolactone (**12**) and isoalloalantolactone (**1**) were all active in promoting root formation on stem cuttings of *P. aureus*. Of these three compounds, alantolactone was distinctly less active than either isoalantolactone or isoalloalantolactone. This showed that the location of an isolated double bond can affect activity.

Significantly, inunal (**6**) displayed considerable activity which was probably due to the presence of an α-methylene-γ-lactone as well as an α,β-unsaturated aldehyde function [8]. In guaianolides, with an α-methylene-γ-lactone, the introduction of an α,β-unsaturated ketone moiety is known to enhance the root forming potential of the α-methylene-γ-lactone moiety [9]. This, however, was not found to be so in the present study since 3-oxoisoalantolactone (**11**) was biologically less potent than its parent isoalantolactone.

EXPERIMENTAL

All the compounds gave a satisfactory C and H analysis. IR: Nujol; ^1H NMR: $CDCl_3$, TMS as int. standard. All the chro-

matographic separations were performed on silica gel impregnated with $AgNO_3$.

Isoalloalantolactone (**1**) *and inunal* (**6**). Powdered *I. racemosa* roots were exhaustively extracted with petrol (40–60°) at room temp. and concd under red. pres. to yield an extract which, on cooling, yielded solid alantolides [alantolactone (**12**) and isoalantolactone (**13**)] and a mother liquor. The mother liquor was chromatographed extensively on silica gel impregnated with $AgNO_3$ and the appropriate fractions containing the minor lactones, free from alantolactones, were rechromatographed and subjected to extensive prep. TLC (10% $AgNO_3$–silica gel G, C_6H_6–EtOAc, 19:1) to afford isoalloalantolactone (**1**) $C_{15}H_{20}O_2$, as a runny liquid (Found: C, 77.64; H, 8.56. $C_{15}H_{20}O_2$ requires: C, 77.55; H, 8.68%.) and alloalantolactone (**4**) IR ν_{max}^{Nujol} cm^{-1}: 1765, 1660, 1645, 1260, 1145, 945, 845, 810; ^1H NMR: δ1.12 (3H, *s*, H-14), 1.67 (3H, *s*, H-15), 4.48 (1H, *m*, H-8), 5.56 and 6.21 (1H each, *d*, $J = 2.5$ Hz, H-13).

The more polar fractions of the oil, after further extensive prep. TLC, afforded inunal (**6**), $C_{15}H_{18}O_3$, mp 160° (Found: C, 72.90; H, 7.55. $C_{15}H_{18}O_3$ requires: C, 73.14; H, 7.37%.) and isotelekin (**10**) IR ν_{max}^{Nujol} cm^{-1}: 3430, 2890, 1725, 1450, 1375, 1257, 895, 820; ^1H NMR: δ0.82 (3H, *s*, H-14), 4.20 (1H, *s*, H-3), 4.42 (1H, *m*, H-8), 4.48 and 4.90 (1H each, *br s*, H-15), 5.49 and 6.02 (1H each, *br s*, H-13).

Tetrahydroalantolactone (**3**). A soln of **1** (1 g) in 50 ml HOAc was hydrogenated over PtO_2 (0.2 g) at 30 lb/in^2 for 2 hr. After removal of the catalyst, by filtration, the solvent was evaporated and the residue crystallized twice from EtOAc–petrol yielding (0.6 g) tetrahydroalantolactone (**3**), mp and mmp (with an authentic sample) 143–144°.

Reaction of **1** *with diazomethane.* A soln of **1** (1.0 g) in Et_2O (20 ml) was allowed to react with an Et_2O soln of CH_2N_2 until the yellow colour persisted for 30 min at room temp. After 24 hr, the solvent was evaporated to afford **2**, $C_{16}H_{22}O_2N_2$, as a colourless liquid. (Found: C, 70.10; H, 8.18. $C_{16}H_{22}O_2N_2$ requires: C, 70.04; H, 8.08%.)

Reaction of **4** *with diazomethane.* A soln of **4** (1.0 g) in Et_2O (20 ml) was allowed to react with an Et_2O soln of CH_2N_2 until the yellow colour persisted for 1 hr at room temp. After 24 hr, a crystalline compound **5**, $C_{16}H_{22}O_2N_2$, mp 145°, separated out. (Found: C, 70.18; H, 7.98. $C_{16}H_{22}O_2N_2$ requires: C, 70.04; H, 8.08%.)

Reduction of inunal. Inunal (1 g) was dissolved in MeOH (10 ml) and $NaBH_4$ (0.5 g) added gradually over 1 hr. The reaction mixture was then kept at the room temp. for 24 hr after which the soln was diluted with H_2O and extracted with Et_2O. The extract was washed, dried and evaporated to yield **8** as a colourless liquid, $C_{15}H_{22}O_3$. (Found: C, 71.84; H, 8.92. $C_{15}H_{22}O_3$ requires: C, 71.97; H, 8.86%.)

Oxidation of **8** *with active manganese oxide.* A soln of **8** (0.5 g) in 10 ml CH_2Cl_2 was stirred with active MnO_2 (2.0 g) for 1 hr at room temp. The mixture was filtered under suction and the ppt washed thoroughly with CH_2Cl_2. The filtrate was evaporated under red. pres. to give a crystalline residue (**9**), $C_{15}H_{20}O_3$, mp 121°. (Found: C, 72.64; H, 8.22. $C_{15}H_{20}O_3$ requires: C, 72.55; H, 8.12%.)

Reaction of **6** *with diazomethane.* A soln of **6** (0.5 g) in Et_2O (10 ml) was allowed to react with an Et_2O soln of CH_2N_2 until the yellow colour persisted for 20 min at room temp. After 1 hr, the excess CH_2N_2 was destroyed and the solvent evaporated. The product was recrystallized from EtOH to yield a crystalline compound (**7**), $C_{16}H_{20}O_3N_2$, mp 143°. (Found: C, 66.75; H, 7.02. $C_{16}H_{20}O_3N_2$ requires: C, 66.64; H, 6.99%.)

Oxidation of **10** *with pyridinium chlorochromate.* A soln of **10** (0.75 g) in 10 ml CH_2Cl_2 was added to a suspension of pyridinium chlorochromate (1.5 g) in 20 ml CH_2Cl_2. The reaction was complete within 2 hr, after which the mixture was filtered. The residual solid was washed thoroughly with Et_2O and the combined filtrate and washings evaporated to give a mixture of two products. The reaction mixture was chromatographed and, on elution with petrol–Et_2O (9:1), afforded **6**, mp 160° (mp, mmp with an authentic sample of inunal). Further elution with petrol–Et_2O (4:1) afforded another crystalline compound, **11**, mp 145°, $C_{15}H_{18}O_3$. (Found: C, 73.05; H, 7.48. $C_{15}H_{18}O_3$ requires: C, 73.14; H, 7.37%.)

Biological testing. For the root initiation study on hypocotyl cuttings of *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 concns (5, 10, 15, 20 mg/l.) along 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 recorded on day 8. The expt was repeated ($\times$ 3) at 27 $\pm$ 2°.

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