Phytochemistry, Vol. 27, No. 7, pp. 2079–2081, 1988.
Printed in Great Britain.

0031–9422/88 $3.00+0.00

EPOXY ALANTOLIDES: ISOINUNAL—A NEW POTENT PLANT GROWTH REGULATOR FROM *INULA RACEMOSA*

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(*Received* 28 *August* 1987)

Key Word Index—*Inula racemosa*; Compositae; alantolides; plant growth regulators; biogenesis; α-methylene-γ-lactone.

Abstract—An investigation of *Inula racemosa* roots afforded, in addition to known sesquiterpene lactones, four new lactones with close biogenetic relationships. Their structures have been elucidated using spectral data and chemical correlation. The most interesting of these is isoinunal which is a potent root initiator with hypocotyl cuttings of *Phaseolus aureus*.

INTRODUCTION

In our search for new plant growth regulators especially sesquiterpenes having the α-methylene-γ-lactone moiety, an extensive investigation of extracts from the powdered roots of *Inula racemosa* is being undertaken. In addition to the detection [1, 2] of alantolactone (**1**) and isoalantolactone (**2**) as the major constituents, we had earlier reported the isolation and structure elucidation [3] of inunal and isoalloalantolactone (**3** and **4**) respectively. The present communication reports the isolation and identification of four more oxygenated alantolides of which isoinunal (**5**) displays considerable root initiation activity with hypocotyl cuttings of *P. aureus*.

RESULTS AND DISCUSSION

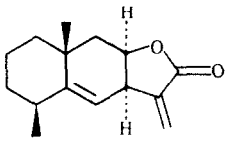
The hexane extract from the powdered roots of *I. racemosa* on cooling gave alantolides which were mainly composed of alantolactone (**1**) and isoalantolactone (**2**). The mother liquor after extensive column chromatography gave four sesquiterpene lactones, in addition to the already reported inunal (**3**) and telekin (**6**). The early fractions of the chromatography yielded a sesquiterpene lactone (**7**), which on spectral analysis showed $[M]^+$ at *m/z* 248.326 ($C_{15}H_{20}O_3$) and a diagnostic fragment ion at *m/z* 232 $[M-16]^+$ indicative of the presence of an epoxide [4]. The characteristic absorption bands at 1770 (γ-lactone) and 1670 cm^{-1} (exocyclic α-methylene) confirmed the presence of an α-methylene -γ-lactone moiety. In its ^1H NMR spectrum (Table 1), it showed a multiplet at δ 4.48 for one hydrogen similar to that given by isoalantolactone (**2**) and related compounds. Indeed, the ^1H NMR spectrum of compound (**7**) was almost identical with that of isoalantolactone, except for the changes due to the presence of an epoxy group in compound **7**. To confirm this, isoalantolactone was treated with perbenzoic acid to yield **7** identical (IR and mmp) with the naturally occurring compound isolated from the oil.

From the same fraction an isomeric epoxide, $C_{15}H_{20}O_3$, mp 166° was isolated. The presence of an epoxide ring at C-5, C-6 and its α-stereochemistry was clear from its ^1H NMR spectrum (δ2.80, *d*, *J* = 1). In accordance with the dihedral angle between 6β-H and 7α-H (~ 85°) $J_{6\beta,7\alpha}$ was almost nil, hence this compound could be represented by **8**. The structure was confirmed by reaction of alantolactone (**1**) with one mol of perbenzoic acid which yielded a solid, mp 160°, identical in all respects with the natural product. Compound **8** was followed by another compound, $C_{15}H_{20}O_3$, mp 217°, whose IR spectrum showed the presence of a hydroxyl group and an α-methylene-γ-lactone moiety (λ_{max} cm^{-1}: 3500, 1760 and 1650). From its ^1H NMR spectral features (Table 1) and the shape and position of a multiplet for one hydrogen (δ 4.92) it was obvious that this compound was an alantolide containing an allylic primary alcohol function (2H, ABq, *J* = 12 Hz). In view of its co-occurrence with epoxide **7**, it was assigned structure **9**. To confirm this, epoxide **7** was treated with dry HCl gas in dry methanol to furnish **9** in good yield (identified by comparative IR spectra).

The less polar fraction of the oil contained inunal (**3**); and on further chromatography over silica gel–silver nitrate (10%) yielded an isomeric compound, isoinunal, $C_{15}H_{18}O_3$, mp 150° having an R_f value very similar to inunal. Its alantolide nature was established by ^1H NMR (4.46, 1H, *m*, characteristic of an alantolide). A downfield one hydrogen singlet at δ 9.8 suggested it was an α,β-unsaturated aldehyde and the absence of a downfield olefinic proton established the tetrasubstituted nature of a double bond. This finding was supported by the UV spectrum (λ_{max} 240.5, ε 8000). Hence compound mp 150° may be represented by (**5**). When **9** was oxidized with active manganese dioxide, it gave **5** in quantitative yield.

Oxidative rearrangement of secondary allylic alcohols with chromium (IV) reagents to afford α,β-unsaturated carbonyl compounds has been reported [6]. These rearrangements have been found useful for effecting the 1,3-transposition of oxygen. A similar rearrangement on telekin of established stereostructure (**6**) was, therefore, expected to afford isoinunal (**5**). Reaction of telekin (**6**)

* Part 25 in the series, 'Terpenoid Plant Growth Regulators'.



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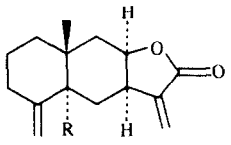
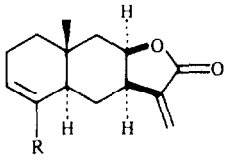
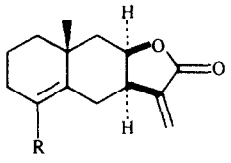


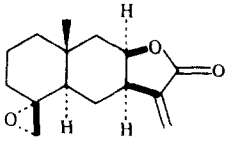
2 R = H
6 R = OH



3 R = CHO
4 R = Me
10 R = CH_2OH



5 R = CHO
9 R = CH_2OH



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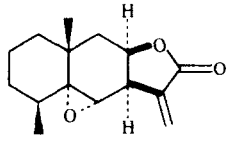

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Table 1. ^1H NMR spectral data of compounds **5–9** (90 MHz, $CDCl_3$, TMS as internal standard, values in parenthesis are coupling constants, Hz)

	IR (ν_{max}^{nujol} cm^{-1})	H-6	H-8	H-14	H-13	H-13′	H-15	H-15′
5	1750, 1710, 1680, 1370 and 890	—	4.46 *m*	1.15 *s*	5.70 *br s*	6.30 *br s*	9.8 *s*	—
7	1770, 1670, 1470, 1385, 1262, 892 and 812	—	4.48 *m*	1.0 *s*	5.53 *br s*	6.15 *br s*	2.5 (5.0) *d*	2.68 (5.0) *d*
8	1765, 1645, 1470, 1272, 942 and 812	2.80(1.0) *d*	4.67 *m*	1.18 *s*	5.73 (3.0) *d*	6.40 (3.0) *d*	1.10 (7.5) *d*	—
9	3500, 1760, 1445, 1392 and 960	—	4.92 *m*	1.21 *s*	5.60 *br s*	6.14 *br s*	4.42 (12.0) Part of ABq	4.55 (12.0) Part of ABq

with pyridinium chlorochromate gave isoinunal (**5**, identical in all respects with the natural product). The co-occurrence of **7**, **9** and **5** and inunal (**3**) together with isoalantolactone suggests that the epoxide **7** might be the biogenetic precursor of isoinunal (**5**) through the intermediacy of compound **9** (a natural product) and also of inunal (**3**) through the intermediacy of **10** which has not yet been isolated.

Structure–biological activity relationship

Biological activity data of most of the compounds have been reported [3,5]. The only new observation is the enhancement in biological activity in inunal (**3**) on its isomerisation to isoinunal (**5**) which is almost thrice as active as the control at 15 and 20 mg/l.

EXPERIMENTAL

Isolation of alantolides. Powdered *Inula racemosa* roots (40 kg) were extracted with petrol (40–60°) at room temp. After removal of the solvent the extract (1.5 kg) was kept at 0° for 4 days to give a solid mass (1.0 kg) of mostly alantolactone and isoalantolactone, and a mother liquor (0.45 kg). The mother liquor (20 g) was chromatographed extensively on silica gel–$AgNO_3$ (10%) and was freed of alantolactone and isoalantolactone by eluting with C_6H_6–EtOAc (19:1). The fractions (3 g) eluted with C_6H_6–EtOAc (10:1) were rechromatographed over silica gel–$AgNO_3$ (10%, 300 g) to afford a pure compound (**8**, 0.2 g), $C_{15}H_{20}O_3$, mp 166°, on elution with petrol–Et_2O (10:1). It analysed for C, 72.08; H, 8.12; $C_{15}H_{20}O_3$ requires: C, 72.28; H, 8.19%. Further elution of the column with the same solvent yielded a solid crystalline compound (**7**, 0.15 g), mp 126°.

It analysed for C, 72.07; H, 8.15, $C_{15}H_{20}O_3$ requires C, 72.28; H, 8.19%. Further elution of the column with the same solvent afforded another crystalline compound (0.1 g, mp 160°), identified as inunal (**3**) by the comparison of its IR and NMR with those of an authentic sample, followed by an aldehyde (**5**, 0.15 g), $C_{15}H_{18}O_3$ mp 150°. Found C, 73.15; H, 7.28, $C_{15}H_{18}O_3$ requires: C, 73.15; H, 7.31%, UV λ_{max}240.5. Elution of the column with petrol–Et_2O (4:1) yielded another pure solid compound (**9**, 0.4 g) mp 217°, $C_{15}H_{20}O_3$. Found C, 72.45; H, 8.01, $C_{15}H_{20}O_3$ requires: C, 72.58; H, 8.06%. IR bands at 3500, 1760, 1445, 1392 and 960 cm^{-1}.

Reaction of compound **7** *with dry* HCl. Compound **7** (0.5 g) was dissolved in dry MeOH and dry HCl gas bubbled through it for 10 min at 0°. After keeping it at 0° for 12 hr. it was diluted with H_2O and extracted with Et_2O. Usual work-up afforded an yellow oil (0.45 g) which was chromatographed over silica gel (25 g). Elution of the column with petrol–Et_2O (4:1) gave a solid compound (0.3 g) identified as **9**.

Oxidation of compound **9** *with active* MnO_2. Compound (**9**, 0.1 g) was dissolved in dry $CHCl_3$ (15 ml) and stirred with active MnO_2 (1 g). After 5 hr at room temp. it was filtered and the solvent evapd to give a solid compound identified as **5**.

Oxidation of compound **6** *with pyridinium chlorochromate.* A soln of **6** (0.8 g) in 10 ml CH_2Cl_2 was added dropwise to a suspension of pyridinium chlorochromate (1.5 g) in 20 ml CH_2Cl_2. The reaction was complete within 4 hr, after which the mixture was diluted with hexane and filtered through a small bed of Al_2O_3 and washed with CH_2Cl_2. The combined organic extracts were evapd and the resulting mixture (0.75 g) was subjected to chromatography over silica gel (5 g). Elution with petrol–Et_2O (4:1) afforded **5** (0.30 g) which showed IR and NMR spectra identical with those of natural product **5**.

Biological testing. For the root initiation studies on hypocotyl cuttings of *P. aureus*, the 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 apex intact. In all four concentrations (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 observation was recorded on day 8. The experiment was repeated three times at $27 \pm 2°$.

Acknowledgements—Th work was financially supported by the Council of Scientific & Industrial Research, New Delhi, India under the scheme, Chemistry of Plant Growth Activity of Terpenoids from Inula Racemosa.

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