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SYNTHESIS AND PROPERTIES OF LUNULARIC ACID*

YOSHITSUGU ARAI, TADAO KAMIKAWA, TAKASHI KUBOTA, YOSHIO MASUDA
and RYOICHI YAMAMOTO
Faculty of Science, Osaka City University, Sugimotocho, Sumiyoshiku, Osaka, Japan

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Key Word Index—Plant growth inhibitor; lunularic acid; *Avena* coleoptile; inhibition of auxin-induced elongation.

Abstract—Lunularic acid, a natural plant growth inhibitor, has been synthesized. A high concentration (10–30 ppm) effectively inhibited the elongation of root coleoptiles caused by 0·3 and 0·03 ppm indole-3-acetic acid.

INTRODUCTION

LUNULARIC ACID (Ia), a natural growth inhibitor from the liverwort *Lunularia cruciata*, has been isolated and characterized.[1] This growth inhibitor appears to occur in all liverworts and all fresh water and marine algae, and is believed to be the biological equivalent of abscisic acid in this group of lower plants.[2–5] In this paper a convenient synthesis of the inhibitor and its biological activity is reported.

RESULTS AND DISCUSSION

The structure of lunularic acid (Ia) was finally confirmed by the hydrogenolysis of hydrangenol (II),[1] a constituent of *Hydrangea macrophylla*.[6] The synthesis of (II), via 7,4′-dimethoxybenzalphthalide (III), has already been reported. Hydrogenolysis of (III) would also give dimethoxy lunularic acid (Ib), however, the yield of (III) from 3-methoxyphthalic anhydride was only 1·5% at best.[7] A more simple preparation would offer advantages over this approach.

Several methods have been investigated for the preparation of 3-arylisocoumarin, which would be converted into dihydrostilbene derivative by hydrogenolysis. Of the methods available, the condensation of homophthalic acid with phenol[8] seems to be attractive. The homophthalic acid (VIIc) has been synthesized by a rather long sequence of reactions,[9] but this route was rejected due to the poor yield. By applying the novel synthesis of 3-methoxyphthalic acid from 1-methoxycyclohexa-1,3-diene (IV),[10] we have obtained 3-methoxyhomophthalic acid.

* Preliminary communication: ARAI, Y., KAMIKAWA, T. and KUBOTA, T. (1972) *Tetrahedron Letters* 1617.

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[4] PRYCE, R. J. (1971) *Phytochemistry* **10**, 2679.

[5] PRYCE, R. J. (1972) *Phytochemistry* **11**, 1759.

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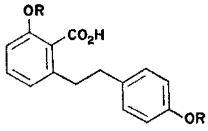
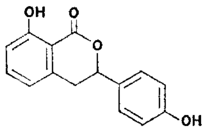


(Ia) R = H
(Ib) R = Me



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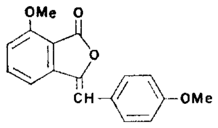

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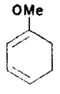

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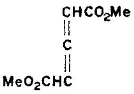

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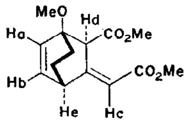
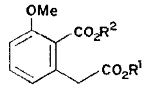
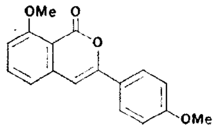


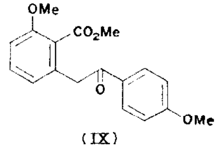
(VI)



(VIIa) $R^1 = R^2 = Me$
(VIIb) $R^1 = H$; $R^2 = Me$
(VIIc) $R^1 = R^2 = H$



(VIII)



(IX)

Heating the mixture of (IV) and dimethyl allene-1,3-dicarboxylate (V)[11] in a sealed tube gave dimethyl 3-methoxyhomophthalate (VIIa), identical with the authentic sample, in 42% yield in one step. The minor product, 5% in yield, of the Diels-Alder reaction was assigned the structure (VI) on the basis of NMR evidence and confirmed by decoupling experiments (Table 1). When the proton H_e was irradiated, an enhancement due to the Nuclear Overhauser effect was observed in the integrated area of H_c proton signals (7·4%). This confirmed the geometry of the conjugated double bond.

TABLE 1. THE NMR DATA OF THE ADDUCT (VI) IN CCl_4

δ	Multiplicity	Assignment	Coupling constants (Hz)
1–2·2	*m*	$\mathrm{H}\ \mathrm{H}_\alpha$ $-\mathrm{C}-\mathrm{C}-\mathrm{C}-\mathrm{C}-\mathrm{H}_e$ $\mathrm{H}_\gamma\,\mathrm{H}_\beta$	
3·06	*m*	H_e	$J_{e,\alpha} = J_{e,\beta} = 3$; $J_{b,e} = 6$
3·40, 3·56 and 3·62	Each *s*	3 OMe	
3·72	*t*	H_d	$J_{c,d} = 2{\cdot}5$; $J_{d,\gamma} = 2$
5·62	*d*	H_c	$J_{c,d} = 2{\cdot}5$
6·08	*dd*	H_b	$J_{a,b} = 9$; $J_{b,e} = 6$
6·36	*d*	H_a	$J_{a,b} = 9$; $J_{a,e} < 1$

Mild acid or base hydrolysis of (VIIa) gave a half ester (VIIb). More vigorous hydrolysis (acid or base) of (VIIa) gave 3-methoxyhomopthhalic acid (VIIc), identical with the authentic sample.[9] Condensation of (VIIc) with anisole in polyphosphoric acid gave an *iso*coumarin (VIII), in 81% yield, which had IR bands at 1710, 1640, 1565 cm^{-1} and UV absorption maximum at 263, 307, 319 and 363 nm. These spectroscopic data together with NMR data are consistent with the 3-arylisocoumarin. When (VIIb) was condensed with anisole, a keto ester (IX) together with (VIII) was obtained in the ratio of about 1:1. Catalytic hydrogenation of (VIII) was rather slow when a small amount of 10% palladium on carbon catalyst was used. However, when a large amount of catalyst was used, the reduction proceeded with moderate speed in nearly quantitative yield to give (Ib). Treatment of (Ib) with BBr_3 afforded (Ia) in 63% yield. The IR spectrum is identical with that

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[11] JONES, E. R. H., MANSFIELD, G H. and WHITING, M. C. (1954) *J. Chem. Soc.* 3208.

of the natural acid and the UV and MS are in accord with the published data. Thus we could obtain lunularic acid in 21 % overall yield in only five steps.

We then observed the effect of lunularic acid on indole-3-acetic acid (IAA)-induced elongation of coleoptile segments excised from 4-day-old etiolated seedlings (*Avena sativa* L., cv. Victory). Seedlings were irradiated with weak red light for 1 day after germination and grown in the dark at 25° thereafter.[12] The apical 2 mm was decapitated from coleoptiles (30 ± 3 mm in length) and 2 hr later a 4 mm segment was excised from the upper region and the first leaf was removed. Coleoptile segments were pooled in buffer solution containing 2 % sucrose, randomized and a group of 12 segments was transferred to 2 ml of test solution with or without 0·3 and 0·03 ppm IAA. The solvent for the test solution was 0·01 M potassium phosphate (pH 5·5) containing 2 % sucrose. After incubation for 3 hr at 25°, the length was determined under a low power (× 20) microscope under dim green light.

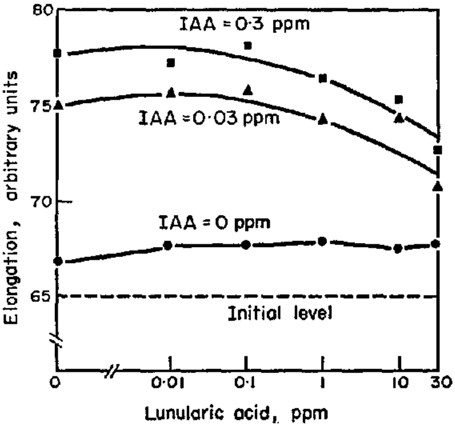

FIG. 1. THE EFFECT OF LUNULARIC ACID ON IAA-INDUCED ELONGATION OF *Avena* COLEOPTILE SEGMENTS. Twelve oat coleoptile segments were incubated for 3 hr in varying concentrations of lunularic acid in the presence or absence of 0·3 and 0·03 ppm IAA.

Figure 1 represents typical results showing that at 10–30 ppm lunularic acid, auxin-induced elongation was effectively inhibited. A double-reciprocal plot showed that lunularic acid at higher concentration (30 ppm) does not inhibit IAA-induced elongation competitively.

EXPERIMENTAL

Unless otherwise stated, the following apply. TLC was performed using Merck silica gel GF_{254} plates with $CHCl_3$–MeOH (19:1) as solvent. NMR spectra were determined on JEOL PS-100 (100 MHz) spectrometer in $CDCl_3$. IR spectra were recorded in Nujol mull with Japan Spectroscopic IR-S spectrophotometer. UV spectra were recorded in 95 % EtOH on Hitachi EPS-2 spectrometer.

Preparation of dimethyl 3-methoxyhomophthalate (VIIa). A mixture of 7·5 g of dimethyl allene-1,3-dicarboxylate (V),[10] 12 g of 1-methoxycyclohexa-1,3-diene (IV)[13] and 100 mg of *p*-hydroquinone was heated at 180° for 17 hr in a sealed tube. The crude product was chromatographed on silica gel. Elution with C_6H_6–AcOEt (19:1) and distillation under vac. gave dimethyl 3-methoxyhomophthalate (VIIa), b.p. 117–124°/0·3 mm Hg (4·73 g). ν_{max} (liquid film) 1740, 1600, 1480, 1460, 1270 and 1070 cm^{-1}, δ (CCl_4) 3·50 (*s*, 2H), 3·54 (*s*, 3H), 3·72 (*s*, 3H), 3·74 (*s*, 3H), 6·70 (*d*, 1H, *J* 8 Hz), 6·72 (*d*, 1H, *J* 8 Hz) and 7·16 (*t*, 1H, *J* 8 Hz). (Found:

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[12] MASUDA, Y. (1968) *Planta* **83**, 171.

[13] BIRCH, A. J. and SUBBA RAO, G. S. (1968) *Tetrahedron Letters* 3797.

C, 60·40; H, 6·08. Calc. for $C_{12}H_{14}O_5$: C, 60·50; H, 5·92%.) Further elution with the same solvent mixture afforded an adduct (VI), for which, after distillation, was obtained a viscous oil, b.p. 116°/0·4 mm Hg (0·587 g). ν_{max} (liquid film) 1730, 1715, 1680, 1650 and 870 cm^{-1}. (Found: C, 62·96; H, 6·75. Calc. for $C_{14}H_{18}O_5$: C, 63·14; H, 6·81%.)

Hydrolysis of (VIIa). (a) *With acid.* A solution of (VIIa; 638 mg) in HOAc (15 ml) and 1 N HCl (15 ml) was heated for 2 hr at 80°. The mixture was evaporated to dryness under vac. and the residue was dissolved in $CHCl_3$. The soln was washed with satd. $NaHCO_3$ soln. The alkaline solution was acidified and extracted with $CHCl_3$. Evaporation of the extract yielded a crystalline half ester (VIIb) (542 mg). Recrystallization from Et_2O–light petrol. gave colorless needles, m.p. 94–95°. ν_{max} 2500–2750, 1720, 1710, 1690 and 1600 cm^{-1}, δ 3·64 (*s*, 2H), 3·81 (*s*, 3H), 3·86 (*s*, 3H), 6·91 (1H, *d*, *J* 8 Hz), 6·98 (1H, *d*, *J* 8 Hz), 7·34 (*t*, 1H, *J* 8 Hz) and 9·43 (broad, 1H). (Found: C, 58·74; H, 5·41. Calc. for $C_{11}H_{12}O_5$: C, 58·92; H, 5·40%.) (b) *With base.* A mixture of crude VIIa (1·97 g) in MeOH (30 ml) and 1 N NaOH (30 ml) was heated at 80° for 25 min. After cooling, the mixture was extracted with $CHCl_3$ and the aqueous phase was acidified with dil. HCl and extracted with $CHCl_3$. Evaporation of the extract yielded the half ester (VIIb), which was recrystallized from Et_2O–light petrol. to give colorless needles, m.p. 93–94° (0·92 g). The above aqueous phase was further extracted with Et_2O to give a small amount of 3-methoxyhomophthalic acid (VIIc), m.p. 159–160° (from EtOH), which was identical with the authentic sample.[9]

Condensation of (VIIb) *with anisole.* A mixture of 1·45 g of (VIIb), 1·45 g of anisole and polyphosphoric acid prepared from 25 g of P_2O_5 and 16·6 ml of 85% H_3PO_4 was stirred at 90° for 50 min. The mixture was cooled and treated with an excess of cold H_2O. The aq. soln was extracted with $CHCl_3$ and the organic layer washed with $NaHCO_3$ soln. The soln was evaporated to dryness to give 1·94 g of neutral products, which were chromatographed on silica gel (60 g). Elution with CH_2Cl_2–AcOEt (19:1) gave a keto-ester (IX) as a pale yellow oil (851 mg). ν_{max} (liquid film) 1730, 1660, 1590 and 1600 cm^{-1}, λ_{max} 287·5 nm (ϵ 16,300), δ (CCl_4) 3·42 (*s*, 2H), 3·37, 3·61 and 3·78 (each *s*, 3H × 3), 6·80 (*m*, 2H), 6·82 (*d*, 1H, *J* 8 Hz), 7·30 (*t*, 1H, *J* 8 Hz), and 7·70 (*m*, 2H). Further elution with the same solvent mixture gave an *iso*coumarin (VIII) (660 mg), m.p. 162–163·5° (from EtOH). ν_{max} 1710, 1640, 1565, 995 and 833 cm^{-1}, λ_{max} 262 (ϵ 19400), 306 (19200), 318 (19300) and 361·5 nm (19700), δ 3·83 (*s*, 3H), 3·98 (*s*, 3H), 6·66 (*s*, 1H), 6·83 (*d*, 1H, *J* 8 Hz), 6·89 (*m*, 2H), 6·92 (*d*, 1H, *J* 8 Hz) and 7·75 (*m*, 2H). (Found: C, 72·33; H, 5·02. Calc. for $C_7H_{14}O_4$: C, 72·33; H, 5·00%.)

Condensation of (VIIc) *with anisole.* A mixture of 1·7 g of (VIIc), 1·7 g of anisole and polyphosphoric acid (prepared from 30 g of P_2O_5 and 20 ml of 85% H_3PO_4) was treated as before. A crude neutral product (2·06 g) was chromatographed on silica gel (60 g). Elution with CH_2Cl_2–AcOEt (19:1) gave the isocoumarin (VIII) as pale yellow prisms (791 mg).

Preparation of lunularic acid (Ia). The isocoumarin (VIII; 390 mg) in 15 ml of glacial HOAc was hydrogenated over 400 mg of 10% C–Pd at room temp. under an atmosphere of H_2 for 24 hr. The catalyst was removed and the filtrate was evaporated under vac. The residue was dissolved in ether which was washed with $NaHCO_3$ soln and dried. Removal of the solvent left a viscous oil, (Ib; 368 mg). To a solution of (Ib; 368 mg) in 10 ml of dry CH_2Cl_2 at −75° was added a soln of BBr_3 (840 mg) in 8 ml of dry CH_2Cl_2. The mixture was kept for 3 hr at −70 to −75° and allowed to warm up to room temp. overnight. The mixture was poured onto ice water and extracted with Et_2O. The Et_2O layer was washed with satd NaCl soln, treated with activated charcoal and dried. Removal of the solvent gave lunularic acid (Ia) as crystals (211 mg), which was recrystallized from Et_2O–light petrol. to give colorless prisms, m.p. 197–198°. The IR spectrum is identical with that of the natural acid and the UV and MS are in accord with the published data.[1]

Acknowledgement—We thank Professor W. W. Schwabe, Department of Horticulture, University of London, for providing us with the IR spectrum of lunularic acid.