Phytochemistry, 1972, Vol. 11, pp. 2295 to 2299. Pergamon Press. Printed in England.

SYNTHESIS AND PLANT GROWTH INHIBITORY PROPERTIES OF (±)-*O*-METHYLXANTHOXIN

R. S. BURDEN, G. W. DAWSON and H. F. TAYLOR

A.R.C. Plant Growth Substance and Systemic Fungicide Unit, Wye College (University of London), Ashford, Kent

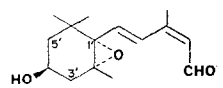
(*Received* 19 *January* 1972)

Key Word Index—Plant growth inhibitor; *O*-methylxanthoxin; abscisic acid; relationship between structure and biological activity; wheat coleoptile growth.

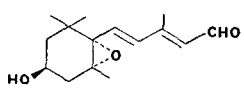
Abstract—The (±)-methyl ethers (XI) and (XII) of *cis,trans*- and *trans,trans*-xanthoxin, a natural plant growth inhibitor, have been synthesized from β-ionone. In the wheat coleoptile section test (±)-*cis,trans*-*O*-methylxanthoxin possesses approximately half the inhibitory activity of the methyl ether of natural *cis,trans*-xanthoxin. This suggests that only one enantiomer of the racemic compound has inhibitory properties.

INTRODUCTION

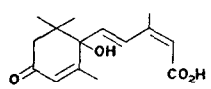
XANTHOXIN is a neutral plant growth inhibitor which can be obtained by oxidative degradation of the pigment violaxanthin;[1,2] it has also been isolated from the ether extracts of many higher plants.[3,4] It was characterized[2,5] largely by spectroscopic methods as a mixture of the isomeric dienals (I) and (II). *cis,trans*-Xanthoxin (I) has activity comparable in many growth tests with abscisic acid (III) and can be converted chemically to this inhibitor.[5] The absolute configuration of xanthoxin is uncertain[2,5] but for the purpose



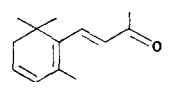
(I)



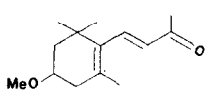
(II)



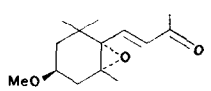
(III)



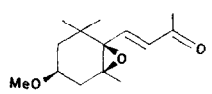
(IV)



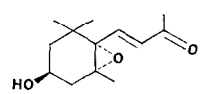
(V)



(VI)



(VII)



(VIII)

of this paper it is assumed to be the same as that proposed[6] for the parent violaxanthin (1′*S*, 2′*R*, 4′*S*).

A synthesis of xanthoxin or a derivative was required to confirm the structure assigned to it and to provide larger quantities of the inhibitor for physiological investigations. In this paper we report our methods leading to a successful synthesis of (±)-*O*-methylxanthoxin.

[1] H. F. TAYLOR and R. S. BURDEN, *Phytochem.* **9**, 2217 (1970).
[2] R. S. BURDEN and H. F. TAYLOR, *Tetrahedron Letters* 4071 (1970).
[3] H. F. TAYLOR and R. S. BURDEN, *Nature, Lond.* **227**, 302 (1970).
[4] R. D. FIRN, R. S. BURDEN and H. F. TAYLOR, *Planta Berl.* **102**, 115 (1972).
[5] H. F. TAYLOR and R. S. BURDEN, *Proc. R. Soc. Lond.* **180B**, 317 (1972).
[6] T. E. DE VILLE, M. B. HURSTHOUSE, S. W. RUSSELL and B. C. L. WEEDON, *Chem. Commun.* 1311 (1969).

The inhibitory activity of this synthetic compound is compared with that of natural xanthoxin and its methyl ether.

RESULTS AND DISCUSSION

A synthesis of xanthoxin was attempted using the readily available β-ionone as starting material, although it was realised that the introduction of an hydroxyl group into the 4′-position was likely to be difficult. 4′-Hydroxy-β-ionone has in fact been recently synthesized[7] but the method used simple precursors and involved many stages with a consequent reduction in overall yield. A more direct preparation from β-ionone would offer advantages over this approach.

3′,4′-Dehydro-β-ionone (IV) was prepared from β-ionone using well established procedures[8,9] and many attempts were made to convert this into 4′-hydroxy-β-ionone by acid catalysed hydration. Hydroboration of the ethylene ketal with a dialkylborane was also attempted but none of these approaches was successful. However, when dehydro-β-ionone was stirred in methanol at 0° in the presence of sulphuric acid,[9] a product was obtained which had a strong IR band at 1098 cm^{-1} consistent with the presence of a methyl ether. The UV-spectrum of this compound was very similar to that of β-ionone and it was concluded that addition of methanol across the 3′,4′-olefinic bond had occurred. At this stage it was not possible to assign the exact location of the methoxy group but stereochemical considerations favoured the C-4′ position (V).

This methyl ether was then reacted with a slight excess of monoperphthalic acid. When treated similarly, β-ionone yields exclusively 1′,2′-epoxy-β-ionone[10] and the corresponding reaction has also been used in carotenoid chemistry to convert zeaxanthin into violaxanthin.[11] In the present case two products were obtained which behaved differently on TLC and GLC. However, their UV and IR spectra were similar and mass spectrometry demonstrated that the compounds were isomers having the molecular formula $C_{14}H_{22}O_3$. The most logical explanation was that the substances had structures (VI) and (VII), and differed only in respect of the relative orientation of the epoxy and methoxy groups.

TABLE 1. GLC* COMPARISON OF SYNTHETIC AND NATURALLY-DERIVED COMPOUNDS

Compound	Column temp. (°)	Retention time (min)
(VI)	165	3·7
(±)-(VI)	165	3·75
(±)-(VII)	165	5·0
(XI), (XII)	195	4·25, 5·0
(±)-(XI), (±)-(XII)	195	4·3, 5·0
(±)-(XV), (±)-(XVI)	195	5·8, 6·8

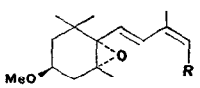
* 150 cm glass column of 2% OV-225 on Gaschrom-Q (nitrogen flow rate 50 ml/min).

[7] D. E. LOEBER, S. W. RUSSELL, T. P. TOUBE, B. C. L. WEEDON and J. DIMENT, *J. Chem. Soc.* **C**, 404 (1971).
[8] U. SCHWEITER, C. V. PLANTA, R. RÜEGG and O. ISLER, *Helv. Chim. Acta* **45**, 528 (1962).
[9] J. D. SURMATIS, *U.S. Pat.* 3354218 (1967).
[10] P. KARRER and H. STÜRZINGER, *Helv. Chim. Acta* **29**, 1829 (1946).
[11] L. BARTLETT, W. KLYNE, W. P. MOSE, P. M. SCOPES, G. GALASKO, A. K. MALLAMS, B. C. L. WEEDON, J. SZABOLCS and G. TOTH, *J. Chem. Soc.* **C**, 2527 (1969).

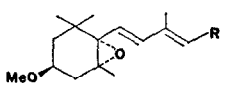
Methylation of the butenone (VIII) prepared by violaxanthin oxidation[1] was achieved with a mixture of methyl iodide, methyl sulphate and calcium oxide in dimethylformamide. The compound obtained was identical by TLC and GLC with one of the above isomers (for GLC comparison, see Table 1). Accordingly, this isomer was assigned the *trans* stereochemistry (VI).

The synthetic butenone (VI) was then subjected to a Horner condensation with trimethylphosphonoacetate in the presence of sodium hydride and tetrahydrofuran, a similar method having been employed[12] in a synthesis of ABA. The product was a mixture of the 2-*cis*,4-*trans* and 2-*trans*,4-*trans* esters (IX) and (X). GLC showed a predominance of the latter isomer but this did not present a serious difficulty as the 2,3 olefinic bond in molecules of this type can be isomerized by sunlight to yield more *cis* isomer.[5,12]

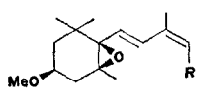
Lithium aluminium hydride reduction of the mixture of *cis,trans* and *trans,trans* esters provided the corresponding alcohols which were then treated with active manganese dioxide in chloroform. The product was shown to be a mixture of the *cis,trans* (XI) and *trans,trans* (XII) isomers of *O*-methylxanthoxin by comparison with the authentic substances obtained by methylation of natural xanthoxin. The *trans,trans*-isomer predominated but after the exposure of an ethyl acetate solution to sunlight for several days, the isomers approached a 1:1 ratio. A GLC comparison of synthetic and natural compounds is shown in Table 1. Attempts at demethylation to give xanthoxin itself have so far proved unsuccessful.



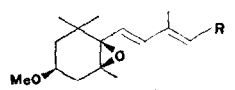
(IX) R = CO_2Me
(XI) R = CHO



(X) R = CO_2Me
(XII) R = CHO



(XIII) R = CO_2Me
(XV) R = CHO



(XIV) R = CO_2Me
(XVI) R = CHO

The isomeric butenone (VII) was treated in the same manner as (VI) to give a product which was not identical with *O*-methylxanthoxin (Table 1). It differed only in having the epoxy and methoxy groups in the *cis* orientation and was clearly a mixture of (XV) and (XVI).

The inhibitory activity of these compounds was assessed in the wheat coleoptile section test where, because of separation difficulties, equivalent mixtures of the 2-*cis*,4-*trans* and 2-*trans*,4-*trans*-isomers were used in all cases. However, in compounds of this type, the *trans,trans*-isomer is believed to make little contribution to the inhibitory response[5,13] and hence the measured activity is largely due to the *cis,trans*-isomer.

The dose-response curves thus obtained for xanthoxin (I, II), natural *O*-methylxanthoxin (XI, XII), synthetic *O*-methylxanthoxin (racemates of XI, XII), and the analogue with the epoxy and methoxy groups *cis* orientated (racemates of XV, XVI) are shown in Fig. 1. This demonstrates that xanthoxin is some four to five times more active in this test than its *O*-methyl derivative, which in turn has activity approximately twice that of the synthetic (racemic) *O*-methyl compound. This latter result was confirmed by repeating the experiment with different samples of natural and synthetic material when the ratio of inhibitory activity was found to be within the limits 1·5 and 3·2. It thus appears that only one enantiomer of (±)-*cis,trans*-*O*-methylxanthoxin is active in this assay. Compounds (XV) and (XVI) showed no significant activity.

[12] J. W. Cornforth, R. Mallaby and G. Ryback, *J. Chem. Soc.* C, 1565 (1968).
[13] B. V. Milborrow, *J. Exptl Bot.* **21**, 17 (1970).

If these results for the methyl ethers can be extrapolated to xanthoxin itself, then it would appear that only the naturally occurring enantiomer is an active inhibitor. Such behaviour would be in marked contrast to that of ABA where both (+)- and (−)-enantiomers are reported to be equally active.[14]

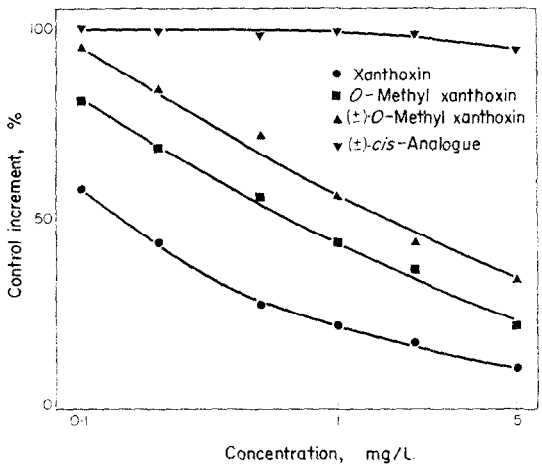


FIG. 1. ASSESSMENT OF INHIBITORY ACTIVITY IN THE WHEAT COLEOPTILE SECTION TEST OF XANTHOXIN (ISOMERS I AND II); *O*-METHYL XANTHOXIN (XI AND XII); (±)-*O*-METHYL XANTHOXIN (RACEMATES OF XI AND XII); AND (±)-*cis*-ANALOGUE (RACEMATES OF XIII AND XIV). THE MEAN INCREMENT OF TWENTY TEST SECTIONS IS EXPRESSED AS A PERCENTAGE OF THE MEAN INCREMENT OF CONTROL SECTIONS.

EXPERIMENTAL

Unless otherwise stated, the following apply. TLC was performed using Merck silica gel F_{254} plates (20 × 20 cm, 0·25 mm thick) with hexane–EtOAc (3:1) as solvent. The various components were located by their quenching characteristics in UV light and in the case of aldehydes and ketones, by spraying with 2,4-dinitrophenylhydrazine reagent. GLC was carried out using a Pye 104 Model 64. UV spectra were determined in EtOH while IR spectra were of liquid films. A GEC-AEI MS902 model was employed for MS. The wheat coleoptile section test was carried out as previously described.[5]

Preparation of (±)-4-(2′,6′,6′-*trimethyl*-4′-*methoxy*-1′-*cyclohexen*-1′-*yl*)-*trans*-3-*buten*-2-*one* (V). Methanol (200 ml) and conc. H_2SO_4 (10 ml) were mixed and stirred at 0° for 30 min under N_2. Dehydro-β-ionone (16 g) was then added and stirring continued for a further 24 hr. Ice-cold water (200 ml) was added to the reaction mixture followed by 50% aq. NaOH (25 ml). Stirring was continued for a further 30 min and the mixture then extracted with light petroleum (b.p. 40–60°, 200 ml), dried (Na_2SO_4), and the solvent removed. The crude product was chromatographed on a column of silicic acid (300 g). Elution with hexane–EtOAc (9:1) solvent afforded 4-(2′,6′,6′-trimethyl-4′-methoxy-1′cyclohexen-1′-yl)-*trans*-3-buten-2-one (V) which, after removal of the solvent, was obtained as a colourless liquid (6·8 g); R_f 0·72; λ_{max} 290 nm; ν_{max} 1678, 1610, 1098 cm^{-1}; M^+ 222 ($C_{14}H_{22}O_2$ requires 222).

Preparation of (±)-4-(trans-1′,2′-*epoxy*-4′-*methoxy*-2′,6′,6′-*trimethyl*-1′-*cyclohexyl*)-trans-3-*buten*-2-*one* (VI) and (±)-4-(cis-1′,2′-*epoxy*-4′-*methoxy*-2′,6′,6′-*trimethyl*-1′-*cyclohexyl*)-trans-3-*buten*-2-*one* (VII). 4-(2′,6′,6′-Trimethyl-4′-methoxy-1′-cyclohexen-1′-yl) *trans*-3-buten-2-one (6·5 g) was dissolved in dry ether (50 ml) to which was added an ethereal solution of monoperphthalic acid (6·5 g, prepared from phthalic anhydride and sodium perborate). The mixture was left at 5° for 48 hr after which it was shaken with saturated aq. $NaHCO_3$ (2 × 100 ml), H_2O (150 ml) and then dried (Na_2SO_4). After evaporation of the solvent, the crude product was chromatographed on a column of silicic acid (140 g). Elution with hexane–EtOAc (7:1) initially gave fractions containing the *trans*-epoxide (VI) followed by fractions of the *cis*-epoxide (VII). After removal of the solvent, the *trans*-epoxide (VI) was obtained as a colourless syrup (2·0 g); R_f 0·50;

[14] P. F. WAREING and G. RYBACK, *Endeavour* **29**, 84 (1970).

λ_{max} 231·5 nm; ν_{max} 1681, 1630, 1098 cm^{-1}; M^+ 238 ($C_{14}H_{22}O_3$ requires 238). The *cis*-epoxide (VII) was similarly obtained as a colourless syrup (2·8 g) which crystallized from light petreoleum as colourless prisms m.p. 58–60°; R_f 0·44; λ_{max} 232·5 nm; ν_{max} 1680, 1630, 1096 cm^{-1}; M^+ 238 ($C_{14}H_{22}O_3$ requires 238).

Preparation of (±)-*methyl* 5-(trans-1′,2′-*epoxy*-4′-methoxy-2′,6′,6′-*trimethyl*-1′-*cyclohexyl*)-3-*methyl*-cis, trans-2,4-*pentadienoate* (IX) *and* (±)-*methyl* 5-(trans-1′,2′-*epoxy*-4′-*methoxy*-2′,6′,6′-*trimethyl*-1′-*cyclohexyl*)-3-*methyl*-trans, trans-2,4-*pentadienoate* (X). Sodium hydride (0·3 g of a 60% dispersion in oil) and dry tetrahydrofuran (10 ml, distilled over $LiAlH_4$) was added dropwise to trimethylphosphonoacetate (1·35 g, prepared from methyl bromoacetate and trimethylphosphite). The thick white slurry obtained was stirred for 30 min and 4-(*trans*-1′,2′-epoxy-4′-methoxy-2′,6′,6′-trimethyl-1′-cyclohexyl)-*trans*-3-buten-2-one (1·5 g) added. The stirring was continued for 24 hr at ambient temperature after which water (100 ml) was added and the mixture extracted with ether (2 × 100 ml). The ether extracts were washed with water, dried (Na_2SO_4) and evaporated. The crude product was purified on a column of silicic acid (50 g) using hexane–EtOAc (8:1) for elution. The purified pale yellow syrup (0·81 g) had λ_{max} 264 nm; ν_{max} 1718, 1634, 1611, 1097 cm^{-1}; M^+ 294 ($C_{17}H_{26}O_4$ requires 294). TLC showed the presence of two components (X) and (IX) (R_f 0·82, 0·73 respectively), the ratio of which was estimated by GLC (2% XE-60, 174°, 50 ml N_2/min flow rate) as 4:1.

Preparation of (±)-*methyl* 5-(cis-1′,2′-*epoxy*-4′-*methoxy*-2′,6′,6′-*trimethyl*-1′-*cyclohexyl*)-3-*methyl*-cis, trans-2,4-*pentadienoate* (XIII) *and* (±)-*methyl* 5-(cis-1′,2′-*exposy*-4′-*methoxy*-2′,6′,6′-*trimethyl*-1′-*cyclohexyl*)-3-*methyl*-trans,trans-2,4-*pentadienoate* (XIV). The corresponding Horner reaction was also carried out with 4-(*cis*-1′,2′-epoxy-4′-methoxy-2′,6′,6′-trimethyl-1′-cyclohexyl)-*trans*-3-buten-2-one (1·5 g). The product was obtained as a viscous yellow liquid (0·83 g), λ_{max} 263 nm; ν_{max} 1718, 1636, 1611, 1098 cm^{-1}; M^+ 294 ($C_{17}H_{26}O_4$ requires 294). Again TLC demonstrated the presence of two components (XIV) and (XIII) (R_f 0·76, 0·67 respectively); GLC indicated an approximate 4:1 ratio.

Preparation of (±)-5-(trans-1′,2′-*epoxy*-4′-*methoxy*-2′,6′,6′-*trimethyl*-1′-*cyclohexyl*)-3-*methyl*-cis,trans-2,4-*pentadienal* (XI) *and* (±)-5-(trans-1′,2′-*epoxy*-4′-*methoxy*-2′,6′,6′-*trimethyl*-1′-*cyclohexyl*)-3-*methyl*-trans, trans-2,4-*pentadienal* (XII). The mixture of esters (IX) and (X) (0·6 g) was dissolved in dry THF (15 ml) and $LiAlH_4$ (0·1 g) added. The mixture was allowed to stand for 1 hr with occasional shaking after which the excess metal hydride was destroyed with a few drops of EtOAc. Water (100 ml) was added and the mixture extracted with ether (2 × 150 ml). After evaporation of the solvent, the mixture of alcohols was dissolved in $CHCl_3$ (50 ml) and shaken overnight with active MnO_2 (6 g). The solution was then filtered, evaporated and the residue chromatographed on a column of silicic acid (25 g) using hexane–EtOAc (3:1) for elution. The purified product was obtained as a colourless syrup (250 mg); λ_{max} 280 nm; ν_{max} 1670, 1631, 1594, 1096 cm^{-1}; no significant molecular ion was observed in the MS. The two isomers were inseparable by TLC (R_f 0·51) but were readily separated by GLC (2% OV-225, 195°, 50 ml N_2/min flow rate). Initially the *trans, trans*: *cis, trans* isomer ratio was 4:1, but after an EtOAc solution of the mixture had been exposed to sunlight for several days, it approached 1:1.

Preparation of (±)-5-(cis-1′,2′-*epoxy*-4′-*methoxy*-2′,6′,6′-*trimethyl*-1′-*cyclohexyl*)-3-*methyl*-cis,trans-2,4-*pentadienal* (XV) and (±)-5-(cis-1′,2′-*epoxy*-4′-*methoxy*-2′,6′,6′-*trimethyl*-1′-*cyclohexyl*)-3-*methyl*-trans, trans-2,4-*pentadienal* (XVI). The mixture of esters (XIII) and (XIV) (0·6 g) was similarly treated with $LiAlH_4$ and MnO_2 to afford a mixture of the aldehydes (XV) and (XVI) as a colourless syrup (210 mg); λ_{max} 281 nm; ν_{max} 1670, 1630, 1592, 1096 cm^{-1}; no significant molecular ion was observed in the MS. Again the isomers could not be separated by TLC (R_f 0·45) but these were readily resolved by GLC (Table 1).

Methylation of xanthoxin (I), (II) *and 'butenone'* (VIII). MeI (2 ml), Me_2SO_4 (0·5 ml), and CaO (0·5 g) were added to a solution of xanthoxin (30 mg, mixture of *cis,trans* and *trans,trans* isomers) in DMF (2 ml). The mixture was stirred and gently heated under reflux for 24 hr. It was then diluted with H_2O (100 ml) and extracted with ether (2 × 150 ml). The ether extracts were washed with 0·1 N aq. NaOH (50 ml), H_2O and dried. Two purifications on TLC with hexane–EtOAc (3:1) solvent afforded *O*-methyl-xanthoxin (XI), (XII) as a colourless syrup (0·8 mg); λ_{max} 281 nm; R_f 0·51. A similar procedure was employed for the methylation of the 'butenone' (VIII) to give the *O*-methyl compound (VI); λ_{max} 231 nm; R_f 0·50.

Acknowledgements—We thank Professor R. L. Wain F.R.S. for his advice and encouragement, and gratefully acknowledge the assistance of Mr. R. P. Townsend (technical aid), Mr. P. Cozens (biological assays), and Mr. R. Self, Food Institute, Norwich (MS).