Synthesis and Geometrical Configuration of Luteoreticulin, a Toxic Nitro-containing Metabolite of *Streptomyces luteoreticuli* Arai

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2,4-Dimethyl-5-(*p*-nitrophenyl)penta-2,4-dienoic acid (2) and the related alcohol (3) and aldehyde (5) have been shown to have the (2*E*,4*E*)-configuration by a nuclear Overhauser effect experiment. The structure and stereochemistry [(6*E*,8*E*)] of luteoreticulin [3-methoxy-2,6,8-trimethyl-9-(*p*-nitrophenyl)nona-2,4,6,8-tetraen-5-olide] (15) have been unequivocally confirmed by synthesis from the acid (2) *via* ring closure of the derived 9-aryl-2,6,8-trimethyl-3,5-dioxonona-6,8-dienoic acid (13).

LUTEORETICULIN,[1,2] a toxic nitro-containing metabolite isolated from *Streptomyces luteoreticuli* Arai together with the biogenetically related aureothin,[3] has been assigned the structure (1),[1] but the geometrical configuration of the conjugated diene system has not been defined hitherto. We describe here a synthesis of luteoreticulin which confirms the structure (1) and establishes the stereochemistry.

It was necessary first to ascertain the geometry of 2,4-dimethyl-5-(*p*-nitrophenyl)penta-2,4-dienoic acid (2),† the starting material, constituting the side-chain at position 5. We therefore undertook a nuclear Overhauser enhancement (n.O.e.) experiment with the corresponding alcohol (3), derived from (*E*)-α-methyl-*p*-nitrocinnamaldehyde (4) [4] by aldol condensation with n-propanal followed by reduction with sodium borohydride of the resulting penta-2,4-dienal (5). On irradiation at the frequency of the 1-methylene protons in (3), an 18% increase was observed in the vinylic 3-proton signal; hence the hydroxymethyl group is *cis* to the vinylic 3-hydrogen atom (*E*-configuration). The geometry of the alcohol (3) and the aldehyde (5) was thus determined as (2*E*,4*E*), and the stereochemistry of the acid (2) was defined as (2*E*,4*E*) by the fact that this acid was obtained by oxidation of the pentadienal (5) with silver oxide. The (2*E*,4*E*)-pentadienoic acid (2) was also conveniently prepared from the aldehyde (4) in 62.3% yield by a Perkin reaction with propionic anhydride and sodium propionate. The acid molecule (2) was extended by one acetate unit,‡ by the method of Bram and Vilkas: [5] the imidazolide of (2) was condensed with the magnesium enol malonate (6) to afford ethyl (4*E*,6*E*)-4,6-dimethyl-7-(*p*-nitrophenyl)-3-oxohepta-4,6-dienoate (7) in 96% yield.§ The n.m.r. spectrum ($CDCl_3$) of the product (7) showed the presence of a 1 : 4 keto–enol mixture. The ketone group in (7) was protected by treatment with ethylene glycol and toluene-*p*-sulphonic acid in benzene to give the acetal (8) (93%), which was then saponified to the acid (9) (95%) with sodium hydroxide in 50% aqueous tetrahydrofuran at room temperature. In order to introduce the propionate unit,[3] compound (9) was subjected to the same procedure as employed in the

† Compound (2) has been obtained from luteoreticulin by degradative oxidation with alkaline hydrogen peroxide.[1] However, its stereochemistry has remained undetermined.

‡ See ref. 3. On ^{13}C and ^{14}C tracer experiments, Yamazaki and his co-workers have demonstrated that luteoreticulin is biosynthesised from three propionate units, one acetate unit, one C_1 unit derived from *S*-methylmethionine, and a remaining undefined C_6–C_1 unit.

§ Corrected yield based on starting material consumed.

[1] Y. Koyama, Y. Fukakusa, N. Kyomura, and S. Yamagishi, *Tetrahedron Letters*, 1969, 359.

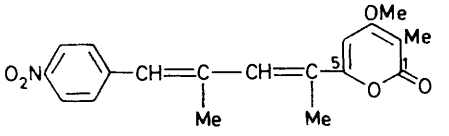
[2] S. Yamagishi, Y. Koyama, Y. Fukakusa, N. Kyomura, J. Ohishi, N. Hamamichi, and T. Arai, *Yakugaku Zasshi*, 1971, **91**, 351.

[3] M. Yamazaki, F. Katoh, J. Ohishi, and Y. Koyama, *Tetrahedron Letters*, 1972, 2701; M. Yamazaki, Y. Maebashi, F. Katoh, Y. Koyama, and T. Tokoroyama, 18th Symposium on the Chemistry of Natural Products, Symposium Papers, Kyoto, 1974, p. 248.

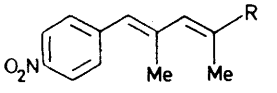
[4] Y. Hirata, H. Nakata, K. Yamada, K. Okumura, and T. Naito, *Tetrahedron*, 1961, **14**, 252.

[5] G. Bram and M. Vilkas, *Bull. Soc. chim. France*, 1964, 945

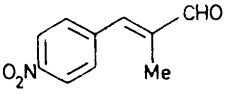
preparation of (7). Thus, the reaction of the imidazolide of (9) with magnesium enol methylmalonate (10) provided the δ-ethylenedioxy-β-oxo-ester (11) (57%) as a viscous oil. This product was then converted into the



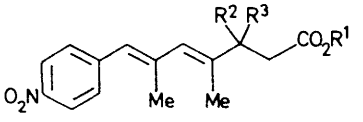
(1)



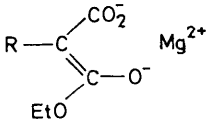
(2) R = CO_2H
(3) R = CH_2OH
(5) R = CHO



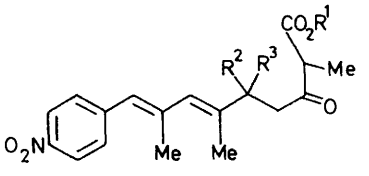
(4)



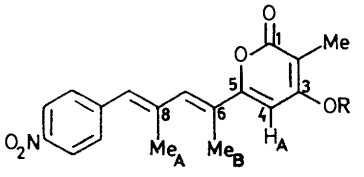
(7) R^1 = Et, R^2R^3 = O
(8) R^1 = Et, R^2R^3 = O·CH_2·CH_2·O
(9) R^1 = H, R^2R^3 = O·CH_2·CH_2·O



(6) R = H
(10) R = Me



(11) R^1 = Et, R^2R^3 = O·CH_2·CH_2·O
(12) R^1 = Et, R^2R^3 = O
(13) R^1 = H, R^2R^3 = O



(14) R = H
(15) R = Me

βδ-dioxo-ester (12) * (86.1%) with a small amount of toluene-*p*-sulphonic acid in acetone under reflux. Subsequent hydrolysis afforded the unstable dioxocarboxylic acid (13), which was immediately cyclized to the desired 4-hydroxy-2-pyrone (14) [70% from (12)] by treatment with acetic anhydride at room temperature. Methylation of 4-hydroxy-2-pyrones can afford two isomeric products, *i.e.* 4-methoxy-2-pyrones and 2-methoxy-4-pyrones.[6] When the pyrone (14) was methylated with dimethyl sulphate and potassium carbonate, the expected luteoreticulin was formed exclusively in 90% yield. The product was identical (mixed m.p., t.l.c., and spectral data) with natural luteoreticulin; that it was in fact the 4-methoxy-2-pyrone derivative (15) was confirmed by the n.O.e. (12%) observed between the methoxy-group and H_A. Moreover, the n.O.e. (22%) between the H_A and Me_B indicates that the conjugated diene system of the side-chain is oriented as depicted in structure (15), and that the stereochemistry is (6*E*, 8*E*).

* Attempts to cyclise the dioxo-ester (12) to the pyrone derivative in several acidic media, *e.g.* trifluoroacetic acid, polyphosphoric acid, concentrated hydrochloric acid, acetic anhydride–sulphuric acid, etc., were unsuccessful. However, when (12) was heated at 140 °C in acetic anhydride, the 4-acetoxy-2-pyrone derivative, m.p. 179—180° (decomp.), was isolated in poor yield; *m/e* 369 (M^+), 326, and 310; δ 1.98 (3 H, s), 2.11br (6 H, s) 2.33 (3 H s), 6.19br (1 H), 6.60br (1 H), and 7.12br (1 H).

EXPERIMENTAL

N.m.r. data were obtained with a Varian A-60 or a JNM-ps-100 instrument for solutions in deuteriochloroform with tetramethylsilane as internal reference. Mass spectra were measured with a Hitachi RMU-6, u.v. spectra (for solutions in ethanol) with a JASCO UVIDEC-1, and i.r. spectra with a JASCO IR-S or IR-G instrument. Column chromatography was carried out on silicic acid (100 mesh; Mallinckrodt).

(2E,4E)-2,4-*Dimethyl*-5-(p-*nitrophenyl*)*penta*-2,4-*dienal* (5).—A solution of propanal (9.12 g) in methanol (60 ml) was added dropwise to a stirred mixture of (*E*)-α-methyl-*p*-nitrocinnamaldehyde (4) [4] (19.1 g), sodium hydroxide (4.8 g), and methanol (600 ml) during *ca.* 3 h at 22 °C. Stirring was continued for 8 h, then water was added slowly over 3 h. The deposited crystals were filtered off, washed with water, and recrystallized from methanol to afford yellow *needles* (5) (5.0 g, 21.7%), m.p. 94—96°; $\nu_{max.}$ (Nujol) 1 663, 1 600, 1 590, 1 505, and 1 343 cm^{-1}; δ 2.03 (3 H), 2.22 (3 H), 6.79 (1 H), 6.86 (1 H), and 9.39 (1 H); $\lambda_{max.}$ 220 nm (ε 11 000), 267sh (8 500), and 331 (20 900) (Found: C, 67.35; H, 5.6; N, 5.6. $C_{13}H_{13}NO_3$ requires C, 67.5; H, 5.65; N, 6.05%).

(2E,4E)-2,4-*Dimethyl*-5-(p-*nitrophenyl*)*penta*-2,4-*dien*-1-ol (3).—To a stirred suspension of the pentadienal (5) (500 mg) in methanol (45 ml), sodium borohydride (62 mg) was added in portions at room temperature. After 25 min the solution was acidified with diluted aqueous acetic acid and most of the methanol was distilled off *in vacuo*. The residual solution was extracted with ether and the extract was washed with aqueous sodium hydrogen carbonate and brine, and dried (Na_2SO_4). Removal of the ether left a viscous oil (496 mg) which was chromatographed on silicic acid in 10 : 1 chloroform–n-hexane. The oily fraction solidified upon cooling and was recrystallized from benzene to give yellow needles, m.p. 56—58°; $\nu_{max.}$ (KBr) 3 390 (OH), 1 595 (C=C), 1 516, and 1 342 cm^{-1} (NO_2); δ 1.87br (1 H, s, removed by D_2O), 1.95br (3 H, d, *J* 1.2 Hz), 2.08br (3 H, d, *J* 1.0 Hz), 4.19 (2 H, m), 6.16br (1 H), and 6.48br (1 H); *m/e* 233 (M^+), 215 (M^+ − 18), 202 (M^+ − 31), and 156 (M^+ − 77).

(2E,4E)-2,4-*Dimethyl*-5-(p-*nitrophenyl*)*penta*-2,4-*dienoic Acid* (2).—*Method A*. The pentadienal (5) (0.312 g) was added gradually to a suspension of silver oxide in aqueous alkali, prepared by the action of silver nitrate (0.75 g) on sodium hydroxide (0.35 g) in water (10 ml). The mixture was heated at 80—85 °C for 1 h with stirring and then filtered. The filtrate was washed with methylene chloride and acidified with concentrated hydrochloric acid to

[6] H. Nakata, *Bull. Chem. Soc. Japan*, 1960, **30**, 1693.

precipitate a pale yellow solid. Recrystallization from tetrahydrofuran gave the *acid* (2) (221 mg, 66%), m.p. 210—211.5° (decomp.) (Found: C, 63.05; H, 5.15; N, 5.55. $C_{18}H_{13}NO_4$ requires C, 63.15; H, 5.3; N, 5.65%); *m/e* 247 (M^+), 230, 202, 156, and 141; $\nu_{max.}$ (Nujol) 1 659, 1 592, 1 515, and 1 344 cm^{-1}. The ethyl ester, m.p. 82—84°; δ 1.35 (3 H, t, *J* 7 Hz), 2.15br (6 H), 4.28 (2 H, q, *J* 7 Hz), 6.67br (1 H), 7.32br (1 H), and 7.48 (2 H, d, *J* 9 Hz); $\lambda_{max.}$ 265 (ε 9 200) and 327 nm (17 600), was prepared by refluxing an ethanolic solution of the acid (2) in the presence of concentrated sulphuric acid.

Method B. A mixture of the aldehyde (5) (22 g), sodium propionate (18.7 g), and propionic anhydride (18.7 g) was heated at 140—145 °C for 25 h, then poured into ice–water. The precipitate was extracted with methylene chloride–saturated aqueous sodium carbonate. The alkaline layer was decolourized with charcoal and acidified with concentrated hydrochloric acid at 0 °C. The deposited solid was filtered off, washed, and recrystallized from tetrahydrofuran to give the acid (2) (17.7 g).

Ethyl (4E,6E)-4,6-*Dimethyl*-7-(p-*nitrophenyl*)-3-*oxohepta*-4,6-*dienoate* (7).—This experiment was essentially carried out by the method of Bram and Vilkas.[5] The acid (2) (13.2 g) was converted into the imidazolide by the action of *NN'*-sulphinyldi-imidazole, prepared from imidazole (14.4 g), thionyl chloride (6.0 g), and tetrahydrofuran (650 ml). To the mixture, a solution of the magnesium enol malonate (6), derived from ethyl hydrogen malonate (8.4 g) and magnesium ethoxide (1 equiv.), was added dropwise below −6 °C with stirring. The mixture was gradually warmed to room temperature and set aside for 3 h. It was then heated at 55 °C for a while, poured into cold 5% hydrochloric acid (180 ml), and extracted with ether. The extract was washed with 5% sodium hydrogen carbonate and brine, dried, and evaporated. The crystalline product was recrystallized from ethanol to give the *oxo-ester* (7) (12.7 g) as yellow needles, m.p. 72—73.5°; δ 1.88 (3 H, t, *J* 7 Hz), 2.05 (3 H, d, *J* 1.5 Hz), 2.12 (3 H, d, *J* 1.5 Hz), 3.80 [0.4 H of C(2)H_2, s], 4.23 (2 H, q, *J* 7 Hz), 5.33 [0.8 H of C(2) H of enol form, s], and 12.30 (0.8 H of 3-OH group of the enol form, s); $\nu_{max.}$ [Nujol) 1 635 and 1 601—1 580 cm^{-1} (β-oxo-ester); $\lambda_{max.}$ 220 (ε 8 400) and 334 nm (13 400); $\lambda_{max.}$ (NaOH–EtOH) 218 (ε 20 000) and 351 nm (11 100) (Found: C, 64.3; H, 6.1; N, 4.4. $C_{17}H_{19}NO_5$ requires C, 64.35; H, 6.05; N, 4.4%). When the alkaline washing was acidified with concentrated hydrochloric acid with cooling, starting material (2) (2.80 g) was recovered.

Ethyl (4E,6E)-3,3-*Ethylenedioxy*-4,6-*dimethyl*-7-(p-*nitrophenyl*)*hepta*-4,6-*dienoate* (8).—A mixture of the oxo-ester (7) (3.30 g), ethylene glycol (2.20 g), toluene-*p*-sulphonic acid monohydrate (100 mg), and benzene (100 ml) was heated under reflux with azeotropic removal of water. After 15 h, the mixture was washed with 5% sodium hydrogen carbonate and brine, dried, and evaporated. The crude oily product was purified by chromatography on silica gel (n-hexane–chloroform, 1 : 5) to give a yellow viscous oil (8) (3.50 g); *m/e* 361 (M^+); $\nu_{max.}$ (Nujol) 1 742, 1 660, 1 595, 1 522, and 1 354 cm^{-1}; δ 2.47 [s, C(2)H_2] and 4.0 (m, O·CH_2·CH_2·O).

(4E,6E)-3,3-*Ethylenedioxy*-4,6-*dimethyl*-7-(p-*nitrophenyl*)-*hepta*-4,6-*dienoic Acid* (9).—A mixture of the acetal ester (8) (2.28 g), sodium hydroxide (0.39 g), and 50% aqueous tetrahydrofuran (80 ml) was stirred at room temperature for 7 h, then shaken with ether several times. From the ethereal layer, starting material (8) (0.80 g) was recovered. The alkaline solution was acidified with 10% hydrochloric acid at 0 °C and then extracted with ether. The extract was washed with brine, dried, and evaporated *in vacuo*. Recrystallization of the residue from benzene afforded the *acetal acid* (9) (1.30 g), m.p. 135—136°, as prisms; $\nu_{max.}$ 1 700, 1 594, and 1 520 cm^{-1}; δ 3.77 [2-C(2)H_2] and 9.62br (CO_2H) (Found: C, 61.65; H, 5.65; N, 4.2. $C_{17}H_{19}NO_6$ requires C, 61.25; H, 5.75; N, 4.2%).

Ethyl (6E,8E)-5,5-*Ethylenedioxy*-2,6,8-*trimethyl*-9-(p-*nitrophenyl*)-3-*oxonona*-6,8-*dienoate* (11).—This compound was prepared as described for compound (7). The imidazolide derived from the acetal acid (9) (1.00 g) was treated with the magnesium enol methylmalonate (10), prepared from ethyl hydrogen methylmalonate (526 mg) and magnesium ethoxide (1 equiv.), to give the acetal oxo-ester (11) (682 mg), as a viscous oil, $\nu_{max.}$ 1 740, 1 717, 1 668, and 1 595 cm^{-1}; δ 1.28 (3 H, t, *J* 7 Hz), 1.39 (3 H, t, *J* 7 Hz), 1.92 (3 H, d, *J* 1.5 Hz), 2.03 (3 H, d, *J* 1.3 Hz), 2.92 (1 H, d, *J* 13.5 Hz), 3.28 (1 H, d, *J* 13.5 Hz), 3.8—4.1 (5 H, m), 6.33br (1 H, s), and 6.43br (1 H, s).

Ethyl (6E,8E)-2,6,8-*Trimethyl*-9-(p-*nitrophenyl*)-3,5-*dioxo*-*nona*-6,8-*dienoate* (12).—A mixture of the acetal (11) (344 mg), toluene-*p*-sulphonic acid monohydrate (27 mg), and acetone (12 ml) was heated under reflux for 5 h, then concentrated almost to dryness. A solution of the residue in ether was washed with 5% sodium hydrogen carbonate and water, dried, and evaporated. Chromatography of the crude product (n-hexane–chloroform, 1 : 10) gave a yellow viscous oil (12) (266 mg); $\nu_{max.}$ (neat) 1 742, 1 663, and 1 595 cm^{-1}.

(6E,8E)-2,6,8-*Trimethyl*-9-(p-*nitrophenyl*)-3,5-*dioxonona*-6,8-*dienoic Acid* (13).—A mixture of the dioxo-ester (12) (373 mg), barium hydroxide octahydrate (630 mg), and 50% aqueous tetrahydrofuran (14 ml) was heated at 75 °C for 1.5 h, concentrated, acidified with 10% hydrochloric acid at 0 °C, and then extracted with ether. The dried extract was concentrated to one-third volume *in vacuo* and shaken with saturated aqueous sodium hydrogen carbonate several times. The combined alkaline solution was acidified with 10% hydrochloric acid at 0 °C and extracted again with ether; the extract was washed with brine, dried ($MgSO_4$), and evaporated *in vacuo* at room temperature to leave an unstable yellow viscous oil (13) (338 mg). Column chromatography caused partial decarboxylation to give a mixture of the acid (13) and (5*E*,7*E*)-5,7-dimethyl-8-(*p*-nitrophenyl)octa-5,7-diene-2,4-dione, m.p. 82—83° (from n-hexane) [1] (Found: C, 67.9; H, 6.5; N, 4.7. Calc. for $C_{17}H_{19}NO_4$: C, 67.75; H, 6.35; N, 4.65%). The crude compound (13) was therefore used immediately for the next experiment.

(6E,8E)-3-*Hydroxy*-2,6,8-*trimethyl*-9-(p-*nitrophenyl*)*nona*-2,4,6,8-*tetraen*-5-*olide* (14).—A mixture of the dioxo-acid (13) (2.00 g) and acetic anhydride (20 ml) was stirred at room temperature. After 6 h, the precipitate was collected and recrystallized from ethyl acetate to give the *pyrone* (14) (1.63 g), m.p. 224—225° (decomp.) (Found: C, 66.4; H, 5.2; N, 4.25. $C_{18}H_{17}NO_5$ requires C, 66.05; H, 5.25; N, 4.3%), $\nu_{max.}$ (Nujol) 1 621, 1 590, 1 565, 1 520, and 1 348 cm^{-1}.

Luteoreticulin (6E,8E)-3-*Methoxy*-2,6,8-*trimethyl*-9-(p-*nitrophenyl*)*nona*-2,4,6,8-*tetraen*-5-*olide* (15).—A mixture of the pyrone (14) (218 mg), dimethyl sulphate (145 mg), potassium carbonate (368 mg), and dry acetone (10 ml) was refluxed for 9 h, cooled, filtered, and concentrated almost to dryness *in vacuo*. A solution of the residue in methylene chloride was washed with brine, dried (Na_2SO_4),

and evaporated; the crystalline product was chromatographed on silica gel (chloroform). The eluant afforded yellow plates of *luteoreticulin* (15) (206 mg), m.p. 184—185° (from ethyl acetate), identical with natural luteoreticulin (Found: C, 67.15; H, 5.7; N, 4.1. $C_{19}H_{19}NO_5$ requires C, 66.85; H, 5.6; N, 4.1%); $\lambda_{max.}$ 226 (ε 23 300), 256sh (11 100), and 368 nm (18 200); δ 1.98 (s, 2-CH_3), 2.10 (d, *J* 1.2 Hz, 8-CH_3), 2.14 (d, *J* 1.2 Hz, 6-CH_3), 3.97 (s, OCH_3), 6.28br (s, 4-H), 6.63br (9-H), 7.18br (7-H), 7.48 (2 H, d, *J* 9 Hz, aromatic), and 8.24 (2 H, d, *J* 9 Hz, aromatic); $\nu_{max.}$ (chloroform) 1 688, 1 635, 1 596, 1 550, 1 519, and 1 346 cm^{-1}; *m/e* 341 (M^+), 326, 298, 139, 83, and 43.

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