Total Synthesis of the Macrolide, (R,S)-Zearalanone†

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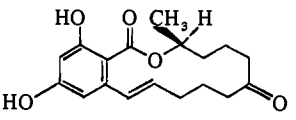
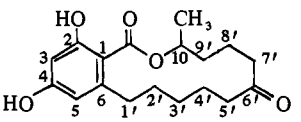
The total synthesis of the macrolide, (R,S)-zearalanone (2), is presented. Estrogen assay of 2 in comparison with (S)-zearalanone (11) derived from a natural source indicates that (R,S)-zearalanone possesses oral uterotropic activity in mice.

The family of lactones related to (S)-zearalanone (1) has been intensively studied in recent years. Isolation of 1 as a metabolite of *Gibberella zeae* and a description of its marked uterotropic and anabolic activities were first reported 10 years ago.[1] Within 4 years the structure of this macrolide had been elucidated.[2] Total synthesis[3] of this structure and determination[4] of its absolute configuration soon followed.



1

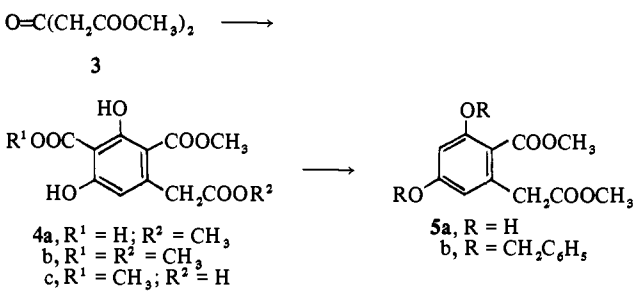


2

We wish to report the total synthesis of a derivative of 1, namely, (R,S)-zearalanone (2). For this synthesis we have developed reactions which we believe have general significance in organic synthesis and particularly in the preparation of polyfunctional macrocyclic structures.[5] These developments have to do with decarboxylations of polyfunctional acids, the Stobbe condensation, and macrocyclizations by the Dieckmann condensation.

Uterotropic assay of 2 in comparison with 1 and (S)-zearalanone (11) showed a measurable level of activity for this racemic material.

Chemistry. Dimethyl β-oxoglutarate (3) was cyclized to 4-carboxy-3,5-dihydroxyhomophthalate (4a). This cyclization, which has been brought about by a variety of metals, metal oxides, metal salts, and organometallic complexes, has been reported to give several products in poor yields.[6,7] In particular, it has been characterized as an unsuitable source of 3,5-dihydroxyhomophthalic acid.[7]



3

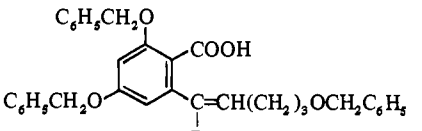
4a, R^1 = H; R^2 = CH_3
b, R^1 = R^2 = CH_3
c, R^1 = CH_3; R^2 = H

5a, R = H
b, R = $CH_2C_6H_5$

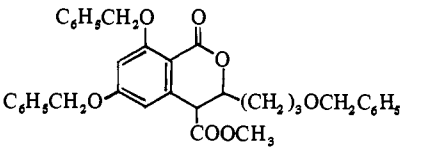
In most reports,[6] cyclization of 3 has led to mixtures of 4a and 4b, with 4b always the major product. In some cases, only 4b has been isolated as a product. We observed that when sodium was used as the condensing agent,[6g] the relative yields of 4a and 4b were quite sensitive to small changes in the amount of sodium present. Whereas with 1% by weight of sodium, it was reported[6g] that 4a and 4b were obtained in 10 and 40% yields, respectively; we found that by using 3% by weight of sodium, the yields reversed to 23 and 14%, respectively. Increasing the amount of sodium used beyond 3% was detrimental to the yield of either product. These results were found to be quite reproducible if careful control of the reaction temperature was exercized. The foregoing yields were obtained in the range 135–140°. Above 140° no 4a was obtained, and the appearance of triester 4b was erratic and in much lower yield.

These results cannot be explained on the basis of partial saponification of 4b to 4a. The aliphatic ester group of a homophthalic ester is more readily saponified than the aromatic group. On treatment with refluxing methanolic potassium hydroxide, 4b gave the aliphatic acid 4c in 87% yield.[6g]

The decarboxylation of 4a to 5a has been reported on several occasions in poor and sometimes unreproducible yields.[8] We found that 5a could be made from 4a in consistently good yields by refluxing in DMF.[5] After benzylation to 5b, the latter was condensed (NaH) with 4-benzyloxybutyraldehyde in an extension[9] of the Stobbe reaction to give a quantitative yield of a mixture of 6a and 7.[5] Dihydroisocoumarin 7 was quantitatively converted to 6a by treatment with sodium methoxide.



6a, R = $COOCH_3$
b, R = COOH
c, R = H



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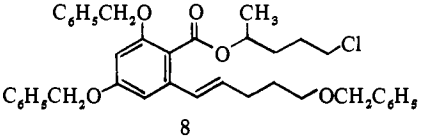
The Stobbe product 6a was readily saponified to diacid 6b, but decarboxylation of the latter to 6c proved troublesome. However, the acid salt of 6b produced 6c smoothly when refluxed in DMF.[5] Reaction of the acid chloride of 6c with 5-chloro-2-pentanol gave 4-chloro-1-methylbutyl



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2,4-bis(benzyloxy)-6-(5-benzyloxy-1-pentenyl)benzoate (8) in good yield. Ester 8 was reduced and hydrogenolyzed to 9a in high yield by treatment with hydrogen at atmospheric pressure with a Pd/C catalyst. Rebenzylation gave alcohol

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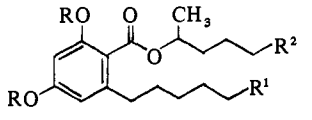
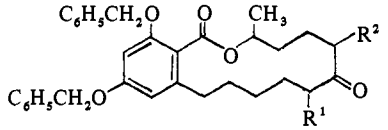


9a, R = H; R^1 = OH; R^2 = Cl
b, R = $CH_2C_6H_5$; R^1 = OH; R^2 = Cl
c, R = $CH_2C_6H_5$; R^1 = R^2 = Cl
d, R = $CH_2C_6H_5$; R^1 = R^2 = CN
e, R = $CH_2C_6H_5$; R^1 = R^2 = $COOCH_3$

9b, which was readily converted to triester **9e** by way of dichloride **9c** and dinitrile **9d**. Preparative yields were readily achieved in all of these steps.

By an internal Dieckmann condensation **9e** was smoothly cyclized in good yield to a mixture of the 2,4-dibenzyl ethers of racemic 5′- and 7′-carbomethoxyzearalanone (**10a** and **10b**) using sodium bis(trimethylsilyl)amide,



10a, R^1 = H; R^2 = $COOCH_3$
b, R^1 = $COOCH_3$; R^2 = H
c, R^1 = R^2 = H

$[(CH_3)_3Si]_2NNa$, as the base.[5] The lactone function of the product is stable under the conditions of this cyclization.

The mixture of **10a** and **10b** was readily hydrolyzed and decarboxylated to the 2,4-dibenzyl ether of racemic zearalanone (**10c**). Hydrogenolysis of **10c** under the same mild conditions used with **9a** gave (*R,S*)-zearalanone (**2**) in good yield.

Biological Evaluation. Uterotropic activities were determined for zearalanone (**11**), obtained from **1** (which has the *S* configuration at the 10′ position),[3] and for synthetic (*R,S*)-zearalanone (**2**), using (*S*)-zearalenone (**1**) as the standard. In this assay **11** showed 1.1 of the activity of the standard (**1**), while **2** exhibited 0.9 of the activity of the standard (**1**). These relative activities represent averages of oral uterotropic activities in mice obtained in two separate series of determinations at three graded dose levels and a control level using six mice for each dosage level for each compound in each series of determinations.

Experimental Section§

The preparation and properties of the following intermediates are described elsewhere as noted: dimethyl 3,5-dihydroxyhomophthalate (**5a**),[5-8] dimethyl 3,5-bis(benzyloxy)homophthalate (**5b**),[5] 6-(5-benzyloxy-1-carbomethoxy-1-pentenyl)-2,4-bis(benzyloxy)benzoic acid (**6a**),[5] α-(4-benzyloxybutylidene)-3,5-bis(benzyloxy)homophthalic acid (**6b**),[5] 3-(3-benzyloxypropyl)-4-carbomethoxy-6,8-bis(benzyloxy)-3,4-dihydroisocoumarin (**7**),[5] 6-(5-benzyloxy-1-pentenyl)-2,4-bis(benzyloxy)benzoic acid (**6c**),[5] 4-chloro-1-methylbutyl 2,4-bis(benzyloxy)-6-(5-benzyloxy-1-pentenyl)benzoate (**8**),[5] 4-chloro-1-methylbutyl 2,4-dihydroxy-6-(5-hydroxypentyl)benzoate (**9a**),[5] a mixture of the 2,4-dibenzyl ethers of racemic 5′- and 7′-carbomethoxyzearalanone (**10a** and **10b**),[5] and the 2,4-dibenzyl ether of racemic zearalanone (**10c**).[5]

Dimethyl β-Oxoglutarate (3). This ester is readily obtained by several processes from citric acid.[10,11] In our hands the best procedure[10] for large-scale preparations consisted of treatment of citric acid with chlorosulfonic acid and then methanol to give a 77% yield of the dimethyl ester.

Dimethyl 4-Carboxy-3,5-dihydroxyhomophthalate (4a) and Dimethyl 4-Carbomethoxy-3,5-dihydroxyhomophthalate (4b). Dimethyl β-oxoglutarate, 100.0 g (0.575 mol), was introduced into a reaction kettle provided with a high-speed stirrer, an air-cooled reflux condenser, a nitrogen line, and efficient means to cool and heat the kettle and its contents. After a nitrogen atmosphere was established, 3.0 g (0.13 g-atom) of finely cut, fresh sodium was added portionwise to the well-stirred contents of the kettle. This reaction was quite exothermic. By cooling and controlling the rate of addition, the temperature was held below 120°.

External heating was applied upon completion of this addition, and the temperature of the stirred reaction solution was maintained at 130–135°. Within about 45 min, the mixture became opaque and began to solidify. Heating and stirring were discontinued, and the reaction mixture was cooled to give 76 g of solid yellow material. This solid was crystallized from methanol to give 23 g of solid, mp 220° dec, which was heated and extracted with 500 ml of water to leave a residue of 7.5 g of water-insoluble triester **4b**, mp 135°. The aqueous extract was acidified (dilute HCl) to precipitate 11.0 g of diester **4a**, mp 148°. The foregoing methanolic filtrate was evaporated to a solid residue which was heated with 200 ml of water. This aqueous phase was filtered from an additional 5.0 g of **4b** and acidified to precipitate another 8.0 g of **4a**. In all, 19.0 g (23.2%) of **4a** and 12.5 g (14.5%) of **4b** were obtained. After recrystallization from methanol **4b** melted at 144° and **4a** at 153°.

Triester **4b** exhibited the following nmr ($CDCl_3$) spectrum: δ 3.69 (s, 3, $-OCH_3$), 3.90 (s, 3, $-OCH_3$), 4.03 (s, 3, $-OCH_3$).

Diester **4a** exhibited the following nmr ($CDCl_3$) spectrum: δ 3.74 (s, 3, $-OCH_3$), 3.97 (s, 3, $-OCH_3$), 14.60–14.81 (s, 1, $-C(=O)OH$). *Anal.* ($C_{12}H_{12}O_8$) C, H, neutral equivalent.

4-Chloro-1-methylbutyl 2,4-Bis(benzyloxy)-6-(5-hydroxypentyl)benzoate (9b). A solution of **9a**[5] (7.00 g) in 150 ml of methyl ethyl ketone, 6.00 g (2.5 molar equiv) of benzyl chloride, and 6.00 g of anhydrous K_2CO_3 was refluxed for 72 hr. Then, after cooling, 50 ml of water was added to the mixture, the ketone layer was separated, and the remaining aqueous layer was extracted twice with 75-ml portions of $CHCl_3$. The ketone and $CHCl_3$ fractions were combined and dried over $MgSO_4$. The solvents were removed to give 9.8 g (83%) of **9b** as a paste: nmr ($CDCl_3$) δ 1.2–1.31 (d, 3, $-OC(CH_3)H$), 5.1–5.3 (m, 1, $-OC(CH_3)H$). *Anal.* ($C_{31}H_{37}O_5Cl$) C, H, Cl.

4-Chloro-1-methylbutyl 2,4-Bis(benzyloxy)-6-(5-chloropentyl)benzoate (9c). **9b** (10.6 g) was dissolved in C_6H_6 together with 4.5 ml of pyridine, the mixture was cooled, and 5.5 g of $SOCl_2$ was added dropwise to it. After stirring the reaction mixture overnight, it was treated with 50 ml of water. The resulting mixture was washed successively with 5% HCl, 5% $NaHCO_3$, and water. After drying ($MgSO_4$), removal of C_6H_6 gave 11.76 g of crude **9c**, which was purified by column chromatography on 150 g of Florisil with $CHCl_3$ to give 9.67 g of pure **9c** (89% yield) as a paste. *Anal.* ($C_{31}H_{36}O_4Cl_2$) C, H; Cl: calcd, 13.05; found, 13.70.

4-Cyano-1-methylbutyl 2,4-Bis(benzyloxy)-6-(5-cyanopentyl)benzoate (9d). **9c** (9.6 g) was dissolved in 20 ml of DMSO, and the solution added dropwise to a solution of 8.50 g of NaCN in 50 ml of DMSO that was warmed to 80°. After addition, the temperature was raised to 120° for 20 min and then kept at 100° overnight. Water (250 ml) was added, and the whole solution was thrice extracted with 200-ml portions of ether. The combined ether extracts were washed with water and dried ($MgSO_4$). Removal of ether gave 9.46 g of crude **9d**, which was purified by passing through 150 g of Silicar-CC-4 with $CHCl_3$ to obtain 7.85 g (85%) of pure **9d** as a paste: nmr ($CDCl_3$) δ 1.9–2.4 (m, 4, two CH_2CN). *Anal.* ($C_{33}H_{36}N_2O_4$) C, H; N: calcd, 5.34; found, 4.71.

4-Carbomethoxy-1-methylbutyl 2,4-Bis(benzyloxy)-6-(5-carbomethoxypentyl)benzoate (9e). **9d** (2.28 g) was added to 7.00 g of HCl gas in 30 ml of dry MeOH, and the mixture was allowed to stand overnight. Water (280 ml) was added, and the mixture was extracted four times with 100-ml portions of ether. The combined ether extracts were washed with 50 ml of 5% $NaHCO_3$ and 50 ml of water and then dried ($MgSO_4$). Removal of ether gave 2.23 g (87%) of **9e**: ir (film) 1725 cm^{-1} (ester C=O, broad band); nmr ($CDCl_3$) δ 1.2–1.3 (d, 3, $-OC(CH_3)H$), 1.38–2.00 (m, 10, $-CH_2CH_2CH_2COOCH_3$ and $-CH_2CH_2CH_2CH_2CH_2COOCH_3$), 2.00–2.8 (m, 6, two CH_2COOCH_3 and benzylic CH_2), 3.68 (d, 6, two CH_2COOCH_3), 5.03 (s, 4, two $OCH_2C_6H_5$), 5.1–5.3 (m, 1, $-OC(CH_3)H$), 6.44 (s, 2, two aromatic H), 7.35 (d, 10, two $OCH_2C_6H_5$).

(*R,S*)-Zearalanone (2). **10c**[5] (90 mg) was dissolved in 15 ml of EtOAc, and 75 mg of 5% Pd/C was added to the solution. The mixture was subjected to atmospheric pressure hydrogenolysis until hydrogenation uptake ceased. After removal of catalyst and solvent a paste was obtained. This was recrystallized from isopropyl alcohol to give 40 mg (69.5%) of **2**, mp 207–208.5°. The ir and nmr spectra are identical with those of **11**: ir (KBr) 3320 (OH stretching, in-

§ Elemental analyses were obtained in our laboratories. Melting points were taken in a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were obtained with a Perkin-Elmer 21 spectrophotometer and nmr spectra with a Varian Associates A-60A spectrometer.

tense), 1685 (ketone C=O, intense), 1630 cm^{-1} (H-bonded ester C=O). *Anal.* ($C_{18}H_{24}O_5$) C, H.

Estrogen Assay. This assay measures uterine weight increase in mice. Adult female CF No. 1 mice were ovariectomized and allowed to recover for 14 days. The compounds were dissolved in sesame oil and administered in three graded doses by gavage for 3 days. On the fourth day the animals were sacrificed, and uteri were removed and weighed.

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Synthetic Luteinizing Hormone Releasing Factor Analogs. Series of Short-Chain Amide LRF Homologs Converging to the Amino Terminus

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The synthesis by solid phase on a benzhydrylamine resin of a series of analogs of the luteinizing hormone releasing factor, LRF, is described. The amidated derivatives of the natural decapeptide LRF (<Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2) described are peptides successively shortened by deletion of 1 (<Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-NH_2), 2, 3, etc., amino acids from the C terminus. These peptide amides were purified by ion-exchange and partition chromatography and were characterized by amino acid analysis, nuclear magnetic resonance spectrometry, and, when possible, mass spectrometry after derivatization. Their specific rotations are reported. Homogeneity of these peptides was tested by thin-layer chromatography in six different solvent systems. The *in vitro* and *in vivo* LRF and follicle stimulating hormone releasing activities and the *in vivo* thyrotropin stimulating hormone releasing activity of these peptides are compared to that of the synthetic LRF and TRF (<Glu-His-Pro-NH_2).

The primary structure of the hypothalamic luteinizing hormone releasing factor (LRF) of porcine[1,2] and ovine[3,4] origins has been demonstrated to be that of the decapeptide <Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2. This peptide stimulates the secretion of the gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), by the anterior pituitary of several species,[5] including man.[6] Synthesis of this decapeptide was started[7] following the Merrifield method on a benzhydrylamine resin first described by Pietta and Marshall[8] and used extensively by us for the synthesis of many peptides.[9] Synthesis of that resin was reported by Monahan, *et al.*,[9] and Rivaille, *et al.*;[10] more details on the chemistry of that resin, as well as a convenient way of controlling its final substitution, will be found in the Experimental Section. Cleavage and deprotection of the peptide is achieved in one step by liquid HF yielding, in the case of LRF, a decapeptide amide showing a single spot in six different tlc systems and which has full biological activity after purification.

Once the methodology was well defined, we undertook the synthesis of the LRF analogs **2–8** in an attempt to find the smallest fragment from the N terminus to have biological activity (Table I).

The protected peptide resins were synthesized in a stepwise manner beginning with a benzhydrylamine resin and using dicyclohexylcarbodiimide[11] (DCI) as the sole coupling agent. The couplings were carried out in CH_2Cl_2 and, in some cases, a mixture of DMF–CH_2Cl_2 (1:1). Thorough washes with MeOH (which contracts the resin) and CH_2Cl_2 (which expands it) to eliminate side products and by-products of the reaction were performed after every coupling step. A ninhydrin test[12] after each coupling was seldom found to be positive; when it was positive, a second coupling with the same BOC amino acid was performed; alternatively, acylation with acetic anhydride in CH_2Cl_2 was employed to

Table I. Synthetic LRF and LRF Analogs

<Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2-(LRF) (1)
<Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-NH_2-des-Gly^{10}-LRF (2)
<Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-NH_2-des-(Pro^9-Gly^{10})-LRF (3)
<Glu-His-Trp-Ser-Tyr-Gly-Leu-NH_2-des-(Arg^8-Gly^{10})-LRF (4)
<Glu-His-Trp-Ser-Tyr-Gly-NH_2-des-(Leu^7-Gly^{10})-LRF (5)
<Glu-His-Trp-Ser-Tyr-NH_2-des-(Gly^6-Gly^{10})-LRF (6)
<Glu-His-Trp-Ser-NH_2-des-(Tyr^5-Gly^{10})-LRF (7)
<Glu-His-Trp-NH_2-des-(Ser^4-Gly^{10})-LRF (8)
<Glu-His-NH_2-des-(Trp^3-Gly^{10})-LRF (9)
<Glu-NH_2-des-(His^2-Gly^{10})-LRF (10)