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Synthesis of Haptens Related to (*Z*)- and (*E*)-Clomiphene

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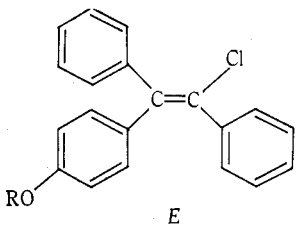
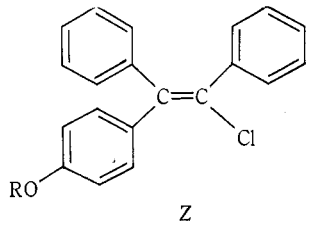
Abstract

Syntheses of (*Z*)- and (*E*)-3-[4-(2-chloro-1,2-diphenylvinyl)phenoxy]propanoic acids (12) and (11), potential haptens for the individual quantitation of the *Z* and of the *E* isomers of clomiphene, 2-[4-(2-chloro-1,2-diphenylvinyl)phenoxy]-*N*,*N*-diethylethanamine (2) and (1), by radioimmunoassay, are described.

Clomiphene citrate, marketed as a mixture of *Z* (2) and *E* (1) isomers, has been extensively applied to the treatment of infertility due to anovulation in patients who have an intact hypothalamic–pituitary axis and responsive ovarian tissue.[1] Its pharmacology, however, has received little attention,[2,3] with no studies on circulating plasma levels, due to a lack of available methodology.



E



Z

(1) R = $CH_2CH_2NEt_2$
(3) R = Me
(5) R = H
(7) R = $CH_2CH{=}CH_2$
(9) R = $CH_2CH_2CH_2OH$
(11) R = $CH_2CH_2CO_2H$

(2) R = $CH_2CH_2NEt_2$
(4) R = Me
(6) R = H
(8) R = $CH_2CH{=}CH_2$
(10) R = $CH_2CH_2CH_2OH$
(12) R = $CH_2CH_2CO_2H$

Radioimmunoassay is an analytical technique of general applicability to the assay of trace organic compounds in biological samples.[4] Extension of those principles

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[2] Schreiber, E., Johnson, J. E., Plotz, E. Z., and Wiener, M., *Clin. Res.*, 1966, **14**, 287.
[3] Schulz, K.-D., Holzel, F., and Bettendorf, G., *Acta Endocrinol. (Copenhagen), Suppl.*, 1967, 119, 226.
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to the development of a clomiphene antigen was limited since clomiphene could not be coupled to a macromolecule such as bovine serum albumin due to a lack of the necessary functional groups, i.e., carboxyl or primary amine. Accordingly it was decided to synthesize an isomerically pure hapten for each of the isomers (1) and (2) so that only the side chain was altered, with the expectation that the raised antibodies would be specific for the individual isomers.

The phenols (6) and (5) were chosen as key intermediates in the preparation of acids (12) and (11). They were synthesized by the following sequence. Addition of anisylmagnesium bromide to benzyl phenyl ketone gave the corresponding alcohol, which was dehydrated by heating with molten sodium bisulfate at 190°. Chlorination of the mixture of isomers was achieved with *N*-chlorosuccinimide/hexamethylphosphoramide; this resulted in a significant improvement in yield of (4) and (3) over that previously reported.[5] Demethylation of (4) and (3) with pyridinium hydrochloride gave the phenols (6) and (5).[6] Isomeric purity, in excess of 95%, of the phenols (6) and (5) was achieved through a unique set of circumstances. The *Z* isomer of the mixture of chloro compounds (4) and (3) crystallized more readily whereas the *E* isomer of the derived mixture of phenols (6) and (5) was the first to crystallize. The stereochemistry of the two geometric isomers was assigned from their u.v. spectra. The *E* isomers of a series of related halogenated stilbenes (whose X-ray crystal structures have been determined) have an absorption at 302 nm whereas the *Z* isomers have an absorption at 292 nm.[7–9]

O-Alkylation of phenols (6) and (5) was surprisingly difficult. Although the anion was generated with sodium hydride in a variety of solvents (dimethoxyethane, dimethyl sulfoxide and tetrahydrofuran), it failed to react with ethyl 3-bromopropionate, ethyl 3-iodopropionate or 1,3-dibromoethane. We were forced, therefore, to use the more reactive allyl bromide. Hydroboration of the allyl ethers (8) and (7) afforded the expected primary alcohols (10) and (9), contaminated with small amounts of secondary alcohols and a significant amount of the boronate esters of phenols (6) and (5). The boronates decomposed, in solution, to their respective phenols (whose structures were confirmed by n.m.r. and by g.l.c.–m.s. of the trimethylsilyl ethers). They result from attack by borane at the 2-position of the allyl ether, followed by a *cis*-elimination of propene; this is a reaction common to allyl ethers.[10] Oxidation of alcohols (10) and (9) with Jones reagent gave the required acids (12) and (11).

Experimental

Melting points were measured on a Reichert hot-stage melting apparatus and are uncorrected. Microanalyses were performed by the Australian Microanalytical Service. ^{1}H n.m.r. spectra were recorded on a Jeol JNM-PHX60 spectrometer (tetramethylsilane as internal standard); i.r. spectra were recorded on a Pye SP1025 spectrometer and u.v. spectra on a Pye SP800 spectrometer. G.l.c.–m.s. was performed on a Hewlett Packard HP5992B fitted with a 2 mm by 2·5 m glass column packed with 2% OV-101 on Chromosorb Q (100/120 mesh) and a membrane separator.

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1-(4-Methoxyphenyl)-1,2-diphenylethanol

A solution of phenyl benzyl ketone (19·7 g, 0·10 mol) in ether (dry, 50 ml) was added during 1 h to a stirred solution of 4-methoxyphenylmagnesium bromide (0·11 mol) in ether (dry, 50 ml). The mixture was boiled under reflux for 2 h, cooled, and quenched cautiously with aqueous ammonium chloride solution (saturated). The mixture was filtered and the ether layer was diluted with an equal volume of hexane. The pale yellow solid which precipitated was filtered, washed repeatedly with hexane and dried, to afford the alcohol (12·6 g, 41%). A sample was recrystallized from ethanol/water as needles, m.p. 112–113° (lit.[11] 112–113°). I.r. (Nujol) ν_{max} 3580, 1615, 1255, 1185, 1035, 835, 815, 710, 700 cm^{-1}.

4-(1,2-Diphenylvinyl)anisole

Alcohol (6·08 g, 0·02 mol) was added portionwise to stirred, molten sodium bisulfate (anhydrous, 20·0 g) at 190°. The mixture was stirred at that temperature for 20 min, cooled, and water (50 ml) and hexane (50 ml) were added. The organic layer was separated, washed with water, dried (Na_2SO_4) and evaporated to afford a mixture of the *Z* and *E* olefins (5·3 g, 93%) as a yellow liquid. ν_{max} (film) 3070, 3040, 1610, 1250, 1180, 1035, 840, 825, 765, 755, 700 cm^{-1}. δ (CCl_4) 7·0–7·3, m, 10H, ArH; 6·9, s, 1H, C=CH; 6·8, ABq, 4H, $MeOC_6H_4$; 3·7, d, 3H, *Z* and *E* OMe.

4-(2-Chloro-1,2-diphenylvinyl)anisole

4-(1,2-Diphenylvinyl)anisole (1·43 g, 0·005 mol), *N*-chlorosuccinimide (2·16 g, 0·0165 mol) and hexamethylphosphoramide (dry, 10 ml) were heated at 100°, under an atmosphere of nitrogen, for 16 h. The mixture was cooled, cyclohexane (20 ml) and water (20 ml) were added, and the cyclohexane layer was separated. The aqueous layer was extracted with a further portion of cyclohexane and the combined cyclohexane extracts were washed with aqueous sodium hydroxide solution (10%) and with water (4 × 20 ml). The cyclohexane solution was dried (Na_2SO_4) and evaporated to afford the vinyl chlorides (4) and (3) (1·60 g, 100%) as a yellow solid, m.p. 76–90°. The two isomers were separated by repeated recrystallization from methanol, the *Z* isomer crystallizing first. In this manner the *Z* isomer was obtained as crystalline needles, m.p. 106·5–107·5° (Found: C, 78·2; H, 5·5; Cl, 10·9. Calc. for $C_{21}H_{17}ClO$: C, 78·6; H, 5·3; Cl, 11·1%). ν_{max} (Nujol) 1610, 1245, 1175, 1105, 1035, 810, 745, 695 cm^{-1}. δ (CCl_4) 7·0–7·3, m, 10H, ArH; 6·7, Abq, 4H, $MeOC_6H_4$; 3·7, s, 3H, OCH_3. The *E* isomer was obtained as crystals, m.p. 121–122°. ν_{max} (Nujol) 1610, 1250, 1175, 1030, 840, 760, 750, 725, 705, 695 cm^{-1}.

4-(2-Chloro-1,2-diphenylvinyl)phenol (6) and (5)

A mixture of vinyl chlorides (4) and (3) (2·0 g, 6·25 mol) and pyridine hydrochloride (2·5 g) was heated at 200° for 2 h, under an atmosphere of nitrogen. The mixture was cooled, water (50 ml) and dichloromethane (50 ml) were added and the organic layer was separated and washed with water (2 × 50 ml). The dichloromethane solution was dried (Na_2SO_4) and treated with charcoal. Evaporation of the filtered solution afforded the phenols (5) and (6) (1·8 g, 94%) as a pale yellow solid. The two isomers were separated by repeated recrystallization from methanol. The *E* isomer crystallized first as crystals, m.p. 128–130°. ν_{max} (Nujol) 3250, 1615, 1175, 755, 705 cm^{-1}. λ_{max} (EtOH) 302 nm (ε 7000). The *Z* isomer was obtained as crystals, m.p. 132–133° (lit.[12] 136°). ν_{max} (Nujol) 3540, 1615, 1025, 805, 750, 700 cm^{-1}. λ_{max} (EtOH) 291 nm (ε 10900). δ (CCl_4) 7·0–7·3, m, 10H, ArH; 6·8, Abq, 4H, C_6H_4OH; 5·0, br s, 1H, OH. *m/z* 308 [M^+ (^{37}Cl), 9%], 306 [M^+ (^{35}Cl), 26%], 165 (39), 73 (100).

3-[4-(2-Chloro-1,2-diphenylvinyl)phenoxy]prop-1-ene (8) and (7)

A solution of the (*E*)-phenol (5) (1·76 g, 5·7 mmol) in dimethoxyethane (dry, 5 ml) was added to a stirred suspension of sodium hydride (1·44 g, 50% dispersion in oil, 30 mmol) in dimethoxyethane (45 ml). The mixture was warmed cautiously to 75° and was stirred at that temperature for 15 min. Allyl bromide (3·63 g, 3·0 mmol) was added to the bright yellow solution, and it was

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heated at 75° for a further 30 min. Water (50 ml) was added cautiously to the cooled mixture and the organic layer was separated, washed with water (2 × 50 ml), dried (Na_2SO_4) and evaporated under reduced pressure to afford a brown liquid (1·78 g). Chromatography of the crude product on Florisil (40 g) with hexane and elution with hexane afforded the (E)-*allyl ether* (7) (1·58 g, 79%) as a solid. A sample was recrystallized from methanol as crystals, m.p. 100–102° (Found: C, 80·1; H, 5·7; Cl, 10·1. $C_{23}H_{19}ClO$ requires C, 79·9; H, 5·5; Cl, 10·0%). ν_{max} (Nujol) 1610, 1245, 1175, 1030, 865, 805, 750, 700 cm^{-1}. *m/z* 348 [M^+ (^{37}Cl), 5%], 346 [M^+ (^{35}Cl), 12], 178 (100), 165 (32).

The (Z)-*allyl ether* (8) was obtained in an identical manner as crystals, m.p. 105–107° (Found: C, 79·5; H, 5·7. $C_{23}H_{19}ClO$ requires C, 79·9; H, 5·5%). ν_{max} (Nujol) 1610, 1240, 1180, 1150, 1025, 860, 830, 755, 700 cm^{-1}. δ (CCl_4) 7·0–7·4, m, 10H, ArH; 6·8, Abq, 4H, ROC_6H_4; 5·9, sym m, 1H, C=CH; 5·3, sym m, 2H, $C=CH_2$; 4·45, sym m, 2H, CH_2. *m/e* 348 [M^+ (^{37}Cl), 6%], 346 [M^+ (^{35}Cl), 13], 178 (100), 165 (34).

3-[4-(2-Chloro-1,2-diphenylvinyl)phenoxy]propan-1-ol (10) and (9)

To a solution of (*E*)-allyl ether (7) (1·4 g, 4·0 mmol) in tetrahydrofuran (dry, 50 ml) was added borane/dimethyl sulfide reagent (1·14 ml, 12·0 mmol). The mixture was heated at 50° for 3 h, cooled, and ethanol (5 ml), aqueous sodium hydroxide solution (10%, 5 ml) and aqueous hydrogen peroxide solution (30% v/v, 5 ml) were added. The tetrahydrofuran was removed by evaporation under reduced pressure and the residual solution was extracted with ether (3 × 20 ml). The combined ethereal extracts were washed with water, dried (Na_2SO_4) and evaporated under reduced pressure, to give an opaque liquid. The crude product was chromatographed on Sorbsil (40 g) with hexane. Elution with hexane/ether (9:1) afforded a small amount of unchanged allyl ether followed by the boronate ether of phenol (5) (376 mg). Elution with hexane/ether (17:3) afforded the (E)-*alcohol* (9) (775 mg, 53%) as needles. A sample was recrystallized from methanol as a solid, m.p. 122–132° (Found: C, 75·8; H, 6·0; Cl, 9·5. $C_{23}H_{21}ClO_2$ requires C, 75·9; H, 5·8; Cl, 9·5%). ν_{max} (Nujol) 3260, 1610, 1250, 1060, 1040, 750, 700 cm^{-1}. δ (CCl_4) 6·9–7·3, m, 10H, ArH; 6·7, ABq, 4H, ROC_6H_4; 3·6–4·2, m, 4H, $2(OCH_2)$; 2·0, sym m, 2H, CH_2; 1·6, s, 1H, OH. *m/z* 366 [M^+ (^{37}Cl), 9%], 364 [M^+ (^{35}Cl), 30], 178 (100), 165 (56).

The (Z)-*alcohol* (10) was prepared in an identical manner as crystals, m.p. 137–139° (Found: C, 75·9; H, 6·1; Cl, 9·6. $C_{23}H_{21}ClO_2$ requires C, 75·9; H, 5·8; Cl, 9·5%). ν_{max} (Nujol) 3430, 1610, 1245, 805, 750, 700 cm^{-1}. *m/e* 366 [M^+ (^{37}Cl), 8%], 364 [M^+ (^{35}Cl), 34], 165 (60), 147 (100).

3-[4-(2-Chloro-1,2-diphenylvinyl)phenoxy]propanoic Acid (12) and (11)

The (*E*)-alcohol (9), in acetone, was oxidized with Jones reagent to afford the (E)-*acid* (11) in 83% crude yield. A sample was recrystallized from chloroform/hexane as needles, m.p. 155–156° (Found: C, 72·9; H, 5·2; Cl, 9·6. $C_{23}H_{19}ClO_3$ requires C, 72·9; H, 5·1; Cl, 9·4%). λ_{max} (EtOH) 302 nm (ε 10800).

The (Z)-*acid* (12) was prepared in an identical manner as needles, m.p. 155–157° (Found: C, 72·6; H, 5·2; Cl, 9·5. $C_{23}H_{19}ClO_3$ requires C, 72·9; H, 5·1; Cl, 9·4%). ν_{max} (Nujol) 1710, 1610, 1245, 1180, 1050, 865, 805, 750, 700 cm^{-1}. δ ($CDCl_3/CD_3SOCD_3$) 6·9–7·3, m, 10H, ArH; 6·8, Abq, 4H, ROC_6H_4; 4·15, sym m, 2H, CH_2O; 2·7, sym m, 2H, CH_2. λ_{max} (EtOH) 299 nm (ε 11400).

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