1:40). The resulting mixture was incubated at 38° for 60 min., then acidified with dilute hydrochloric acid, and subsequently evaluated by paper chromatography on solvents 4 and 5, utilizing ninhydrin and $FeCl_3$–$K_3Fe(CN)_6$ reagents as indicators. The octapeptide remained essentially unchanged, only traces of two new spots could be observed; no phenylalanine could be detected. (The hexapeptide H·L-val-L-tyr-L-val-L-pro-L-phe·OH under identical conditions was digested quantitatively, releasing phenylalanine.)

Chymotrypsin.—The octapeptide (**3**, 1 mg.) was dissolved in 0.20 ml. of pH 8.6 buffer and 0.016 ml. of a solution of 0.25% crystalline chymotrypsin (Worthington) was added (enzyme–substrate, 1:25). After incubation for 1 hr. at 38°, the reaction mixture was acidified with dilute hydrochloric acid and then assayed by paper chromatography on solvents 4 and 5, utilizing *p*-nitrobenzenediazonium fluoroborate and $FeCl_3$-$K_3Fe(CN)_6$ reagents as indicators. The octapeptide (**3**) was digested quantitatively to the tetrapeptide L-valyl-L-histidyl-L-prolyl-L-phenylalanine, R_f (solvent 4) 0.33 and R_f (solvent 5) 0.43 (yellow reaction with *p*-nitrobenzenediazonium fluoroborate), and to another component, presumably D-aspartyl-L-arginyl-L-valyl-L-tyrosine, R_f (solvent 4) 0.15 and R_f (solvent 5) 0.11, which gave a purple spot with the same reagent and a positive reaction with the $FeCl_3$–$K_3Fe(CN)_6$ spray [L-valyl-L-histidyl-L-prolyl-L-phenylalanine amide has R_f (solvent 4) 0.74, R_f (solvent 5) 0.37].

Trypsin.—The octapeptide (**3**, 2 mg.) was dissolved in 0.20 ml. of 0.05 *N* buffer (pH 8.6), 0.016 ml. of a solution of 0.25% crystalline trypsin (Worthington, 3x crystallized) was added, and the solution was incubated at 38° for 1 hr. (enzyme–substrate, 1:50). The reaction mixture was then acidified and assayed by paper chromatography. The peptide was digested quantitatively to L-valyl-L-tyrosyl-L-valyl-L-histidyl-L-prolyl-L-phenylalanine amide, R_f (solvent 3) 0.65, R_f (solvent 4) 0.85 [positive reactions with $FeCl_3$–$K_3Fe(CN)_6$ and *p*-nitrobenzenediazonium fluoroborate sprays].

Leucine Aminopeptidase.—The enzyme (Worthington) was activated before use for 3 hr. at 40°. The activation mixture consisted of 0.20 ml. of 0.025 *M* $MnCl_2$ solution, 0.50 ml. of pH 8 buffer, 0.50 ml. of water, and 0.20 ml. of 0.5% leucine aminopeptidase solution.

The octapeptide (2 mg.) was added to 0.2 ml. of the above activated leucine aminopeptidase, the resulting solution was incubated at 38° for 24 hr. and subsequently evaluated by paper chromatography. No degradation could be detected with indicators ninhydrin and *p*-nitrobenzenediazonium fluoroborate. (The hexapeptide, L-valyl-L-tyrosyl-L-valyl-L-histidyl-L-prolyl-L-phenylalanine, under identical conditions was digested quantitatively to valine, tyrosine, and the tripeptide, L-histidyl-L-prolyl-L-phenylalanine.)

Biological Determinations.—Asparaginyl[1]-valyl[5] angiotensin II[6,10] was used as a standard in all preparations.

Rat Uterus.—Virgin Wistar strain rats (180–200 g.) were injected intramuscularly with diethylstilbesterol, 0.1 mg./kg., 20 hr. before being sacrificed. Uterine strips were suspended in 40 ml. of DeJalon's solution maintained at 30°, and aerated with a mixture of 95% O_2 and 5% CO_2. The peptides were allowed to act for 2 min. before washing.

Guinea Pig Ileum.—Segments of terminal ileum, 4 cm. long, were excised from male Hartley strain guinea pigs (290–310 g.) and suspended in 40 ml. of Tyrode solution maintained at 38° and aerated with 95% O_2 and 5% CO_2. The peptides were allowed to act for 2 min. before washing.

Blood Pressure Measurements.—The procedures followed were essentially those described by Gross and Lichtlen.[20]

(19) Hypertensin®, Ciba. This substance produced an average increase of 50 mm. in rat blood pressure of intact phenobarbital-anesthetized rats following intravenous administration of 0.1–0.2 γ/kg.

(20) F. Gross and P. Lichtlen, *Arch. exptl. Pathol. Pharmakol.*, **233**, 323 (1958).

Synthetic Antigonadotropins. I. Triarylethylenes

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A preliminary series of triarylethanol and triarylethylene compounds was prepared and tested for hormonal and antihormonal effects. Beyond some weak estrogenic effects, the triarylethanols showed no activity. Some of the triarylethylenes proved to be very active antigonadotropins.

The Triarylethylenes.—In an attempt to make a pyridine analog of triparanol, 1-(*p*-methoxyphenyl)-1-(4-pyridyl)-2-(*p*-chlorophenyl)ethanol (I) was prepared from *p*-methoxyphenyl 4-pyridyl ketone and *p*-chlorobenzyl chloride by means of a Grignard reaction. Treatment with hydrobromic acid to hydrolyze the methoxy group also served to split out water from the aliphatic moiety to produce 1-(*p*-hydroxyphenyl)-1-(4-pyridyl)-2-(*p*-chlorophenyl)ethylene (XX). The resemblance between the latter and other compounds known to have marked estrogenic and other hormone-like effects suggested that an excursion into this area might be profitable. The present report concerns some preliminary findings resulting from this excursion.

With the single exception already noted all of the triarylethylenes prepared in this series were made by substantially the same method. The appropriate ketones and Grignard reagents reacted to give the triarylethanols which, on dehydration, gave the desired triarylethylenes.

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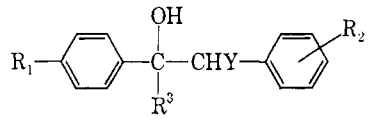
Tables I and II list the triarylethanols and the triarylethylenes prepared in this series. Two isomeric pairs are listed in Table II, namely, XXI and XXII, and XXXIX and XL. The configurations of these compounds have not been determined.

Pharmacology.—The antigonadotropic activity was determined in intact immature male rats. The effect of oral administration of the compounds in oil, given once each day for 10 working days, on the weights of the testes and the prostate was compared to that of the controls and expressed as the per cent difference from the controls. The estrogenic activity was determined on intact immature female rats given subcutaneous injections of the compounds in oil for 3 consecutive days. The increase in uterine weight of the treated animals was compared to that of the controls and was expressed as the per cent difference from the controls.

Beyond weak estrogenic effects, the triarylethanols have so far shown no notable activity. On the other hand, some of the triarylethylenes have shown very marked antigonadotropic effects in a preliminary screen-

TABLE I

TRIARYLETHANOLS

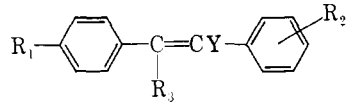


Compd. no.	R_1	R_2	R_3	Y	M.p., °C.	Calcd., % C	Calcd., % H	Calcd., % N	Found,[a] % C	Found,[a] % H	Found,[a] % N
I	CH_3O	*p*-Cl	4-Py	H	229–230	74.2	4.6	4.6	74.4	5.0	4.3
II	$(Et)_2NC_2H_4O$	*p*-Cl	4-Py	H	138–138.5	70.7	6.9	6.6	71.1	6.9	6.6
III	$C_6H_5CH_2O$	*p*-Cl	4-Py	H	213–214	75.2	5.3	3.4	74.9	5.5	3.6
IV	H	*p*-Cl	4-Py	H	180–181	73.6	5.2	...	73.5	5.2	...
V	C_6H_5	H	4-Py	H	203–204	85.4	6.0	...	85.6	5.9	...
VI[b]	C_6H_5	H	C_6H_5	H	132.5–133.5						
VII	CH_3O	*o*-F	C_6H_5	H	122–123	78.1	5.9	...	78.3	6.0	...
VIII	CH_3O	*p*-F	C_6H_5	H	124–125	78.1	5.9	...	78.3	6.0	...
IX	$(Et)_2NC_2H_4O$	*p*-F	4-Py	H	142–143	73.4	7.1	...	73.1	7.0	...
X	$(Et)_2NC_2H_4O$	*o*-F	4-Py	H	134–135	73.4	7.1	6.9	73.6	7.0	6.7
XI	CH_3O	H	C_6H_5	CH_2	107.5–108.5	83.0	6.9	...	82.8	7.1	...
XII[c]	H	H	C_6H_5	CH_2	87–88	87.4	7.4	...	87.1	6.7	...
XIII	H	*p*-F	C_6H_5	H	88–89	82.2	5.8	...	82.2	6.0	...
XIV	H	*o*-F	C_6H_5	H	89.5–90.5	82.2	5.8	...	82.5	6.1	...
XV[d]	H	H	C_6H_5	H	87–88						
XVI	$(Et)_2NC_2H_4O$	*p*-Cl	4-Py	H	123–125	70.4	6.6	6.8	70.7	6.8	6.9
XVII	H	*p*-Cl	2-Py	H	148–148.5	73.6	5.2	4.5	73.6	5.2	4.4
XVIII[e]	CH_3O	H	C_6H_5	H	111–112						
XIX	CH_3O	*p*-Cl	C_6H_5	H	117–118	74.6	5.6	...	74.5	6.0	...

[a] Microanalysis were performed by Dr. A. Steyermark and staff of these laboratories. [b] W. Schlenk and E. Bergmann, *Ann.*, **464,** 29 (1928). [c] A. W. Fort and J. D. Roberts, *J. Am. Chem. Soc.*, **78,** 584 (1956). [d] C. Hell and F. Wiegandt, *Ber.*, **37,** 1431 (1904). [e] C. F. Koelsch, *J. Am. Chem. Soc.*, **54,** 2490 (1932).

TABLE II

TRIARYLETHYLENES



Compd. no.	R_1	R_2	R_3	Y	B.p. (mm.) or m.p., °C.	Calcd., % C	Calcd., % H	Calcd., % N	Found, % C	Found, % H	Found, % N
XX	OH	*p*-Cl	4-Py	H	229–230	74.2	4.6	4.6	74.4	5.0	4.3
XXI	CH_3O[a]	*p*-Cl	4-Py	H	180–181	62.8	4.5	3.3	62.8	4.6	3.7
XXII	CH_3O[b]	*p*-Cl	4-Py	H	161–163	64.0	4.4	8.6	63.9	4.8	7.9
XXIII	$(Et)_2NC_2H_4O$[c]	*p*-Cl	4-Py	H	*ca.* 100	58.1	5.9	4.8	57.6	6.1	4.7
XXIV[g]	H[d]	*p*-Cl	C_6H_5	H	60–61	82.6	5.2	Cl, 12.2	82.7	5.4	Cl, 12.4
XXV	H	*p*-Cl	4-Py	H	96–97	78.2	4.8	4.8	78.4	5.0	4.8
XXVI[h]	C_6H_5	H	C_6H_5	H	132.5–133.5						
XXVII	C_6H_5	H	4-Py	H	140–141	90.0	5.7	...	90.3	5.6	...
XXVIII	$(Et)_2NC_2H_4O$	*o*-F	4-Py	H	210–215 (0.2)	77.0	6.9	6.0	77.4	7.1	...
XXIX[i]	H	H	C_6H_5	H	69–70						
XXX	CH_3O	*o*-F	C_6H_5	H	190–195 (0.4)	82.8	5.6	...	83.0	5.9	...
XXXI	CH_3O	*p*-F	C_6H_5	H	182–185(0.25)	82.8	5.6	...	82.8	5.6	...
XXXII	$(Et)_2NC_2H_4O$	*p*-F	4-Py	H	...	77.0	6.9	7.2	76.2	6.9	7.2
XXXIII	CH_3O	H	C_6H_5	CH_2	70–71	88.0	6.7	...	87.9	6.9	...
XXXIV	H	*p*-F	C_6H_5	H	58–59	87.6	5.5	...	87.8	5.5	...
XXXV	H	*o*-F	C_6H_5	H	62–63	87.6	5.5	...	87.9	5.5	...
XXXVI	H	H	C_6H_5	CH_2	155–160 (0.2)	93.3	6.7	...	92.7	7.0	...
XXXVII[j]	CH_3O	H	C_6H_5	H	192–195 (0.45)	88.2	6.3	...	88.2	6.8	...
XXXVIII	CH_3O	*p*-Cl	C_6H_5	H	200–205 (0.25)	78.8	5.3	...	78.5	5.7	...
XXXIX	H[e]	*p*-Cl	2-Py	H	207–209	61.2	4.0	3.8	61.1	4.1	3.9
XL	H[f]	*p*-Cl	2-Py	H	254–256	61.2	4.0	3.8	61.8	4.1	4.2

[a] Isomeric with XXII, melting point and analysis of $C_{20}H_{16}ClNO \cdot H_2C_2O_4 \cdot 0.5H_2O$: Cl, 8.4. Found: Cl, 8.6. [b] Isomeric with XXI, melting point and analysis of $C_{20}H_{16}ClNO \cdot H_2C_2O_4$. [c] Melting point and analysis of $C_{25}H_{27}ClN_2O \cdot 1.5H_2C_2O_4 \cdot 2H_2O$. [d] W. T. Tadros, K. Farahat, and J. M. Robson [*J. Chem. Soc.*, 439 (1949)] reported m.p. 76–77°. [e] Isomeric with XL, melting point and analysis of $C_{19}H_{14}ClN \cdot HBr$. [f] Isomeric with XXXIX, melting point and analysis of $C_{19}H_{14}ClN \cdot HBr$. [g] See footnote *d*, Table II. [h] See footnote *b* Table I. [i] See footnote *d* Table I. [j] See footnote *e* Table I.

ing. The most active of these are listed in Table III. In addition to the antigonadotropin activity, Table III records the estrogenic activity to show the magnitude of this undesirable component.

Experimental

All melting points are corrected; all boiling points are uncorrected.

General Methods. The Triarylethanols.—To the Grignard

reagent made by interacting slightly more than 1 equiv. of magnesium with slightly more than 1 equiv. of the appropriate aralkyl halide in ether was added, in ether solution, 1 equiv. of the appropriate ketone. The reaction mixture was generally permitted to stir at room temperature for 1 hr. On occasion, it was found desirable to reflux the mixture for 1 hr. At the end of this time, the Grignard reagent was destroyed with water or methanol, the latter being imperative in some instances. The triarylethanol product was generally isolated from the ether phase. Those compounds with basic nitrogen atoms were converted to appropriate salts for convenience in isolation and purification. In some cases, tetrahydrofuran was substituted for ether in order to obtain satisfactory yields.

Table III

Antigonadotropins

Compd. no.	Activity (50 mcg./kg./day), ——% change—— Testes	Prostate	Estrogenic activity (5 mg./rat/day), % change in uteros
XXIV	−60	−59	+176[a]
XXIX	−70	−85	+148
XXX	−75	−80	+180
XXXI	−58	−59	+158
XXXIV	−70	−77	+178
XXXV	−68	−79	+230
XXXVII[b]	−68	−79	+256
XXXVIII	−44	−44	+142

[a] Dose, 3 mg./rat/day. [b] See footnote c, Table I.

The Triarylethylenes.—The conversion of the triarylethanols to the triarylethylenes was generally effected by several hours refluxing with 20% 2-propanolic hydrogen chloride. In some instances, the dehydration was achieved by distillation of the alcohol with or without a few drops of sulfuric acid. Only rarely was 48% hydrobromic acid required. The basic compounds were generally isolated and purified as salts. The neutral compounds were purified by distillation. To illustrate the above general methods, the synthesis of the new and active compounds in this series are presented in detail.

1-(*p*-Methoxyphenyl)-1-phenyl-2-(*o*-fluorophenyl)ethylene (XXX).—To 1.4 g. of magnesium in 15 ml. of ether was added dropwise, with stirring, 9 g. of *o*-fluorobenzyl chloride in 10 ml. of ether. After the addition was complete, the mixture was stirred until the magnesium was in solution. To the mixture was then added dropwise, with stirring, a solution containing 10 g. of *p*-methoxybenzophenone in 40 ml. of ether. The reaction mixture was stirred for about 1 hr. and 10–15 ml. of methanol was then added. The mixture was filtered and the filtrate, on evaporation of the ether, yielded 14.5 g. of 1-(*p*-methoxyphenyl)-1-phenyl-2-(*o*-fluorophenyl)ethanol (VII). Upon recrystallization from methanol, it was obtained in the form of white needles which melted at 122–123°.

Five grams of the alcohol was refluxed with 75 ml. of 20% 2-propanolic hydrogen chloride for 7 hr. The solvent was then removed and the residual oil distilled. The product was obtained as a viscous, yellow oil which distilled at 190–195° (0.4 mm.).

1-(*p*-Methoxyphenyl)-1-phenyl-2-(*p*-fluorophenyl)ethylene (XXXI).—The procedure described for XXX was repeated exactly, using *p*-fluorobenzyl chloride in place of the *ortho* isomer to give 14.5 g. of 1-(*p*-methoxyphenyl)-1-phenyl-2-(*p*-fluorophenyl)-ethanol (VIII) which on recrystallization from methanol was obtained in the form of small white needles melting at 124–125°.

Five grams of the alcohol was treated with 20% 2-propanolic hydrogen chloride as described for XXX. On distillation, the product was obtained as a viscous, colorless liquid, b.p. 182–185° (0.25 mm.).

1,1-Diphenyl-2-(*p*-fluorophenyl)ethylene (XXXIV).—To 2.2 g. of magnesium in 20 ml. of ether was added, dropwise and with stirring, 19 g. of *p*-fluorobenzyl chloride in 20 ml. of ether. Upon solution of the magnesium, 20 g. of benzophenone in 80 ml. of ether was added and the mixture was refluxed for 1 hr. On the addition of methanol, filtration, and removal of the ether, a crystalline product was obtained which was recrystallized from Skellysolve B to give 10.5 g. of colorless prisms of 1,1-diphenyl-2-(*p*-fluorophenyl)ethanol (XIII), melting at 88–89°.

Five grams of the above alcohol was refluxed overnight with 70 ml. of 20% 2-propanolic hydrogen chloride. The solvent was evaporated under vacuum and the residue was recrystallized from 2-propanol to give 4.5 g. of the product in the form of granular clusters which melted at 58–59°.

1,1-Diphenyl-2-(*o*-fluorophenyl)ethylene (XXXV).—The procedure described for the *p*-fluoro derivative of XXXIV was repeated with 2.2 g. of magnesium, 18 g. of *o*-fluorobenzyl chloride, and 20 g. of benzophenone to give 10 g. of 1,1-diphenyl-2-(*o*-fluorophenyl)ethanol (XIV) in the form of fine white needles (from Skellysolve B) which melted at 89.5°.

Three grams of the alcohol was dehydrated and worked up as described for the *p*-fluoro derivative of XXXIV and gave 2.5 g. of the product in the form of colorless plates which melted at 62–63°.

1-(*p*-Methoxyphenyl)-1-phenyl-2-(*p*-chlorophenyl)ethylene (XXXVIII).—To 2.4 g. of magnesium in 20 ml. of ether was added dropwise with stirring 16 g. of *p*-chlorobenzyl chloride in 20 ml. of ether. When the magnesium had dissolved, 15 g. of *p*-methoxybenzophenone in 40 ml. of ether was added portionwise. The reaction mixture was stirred for about 1 hr. and 15 ml. of methanol was then added. The mixture was filtered and the ether removed from the filtrate to give 25 g. of 1-(*p*-methoxyphenyl)-1-phenyl-2-(*p*-chlorophenyl)ethanol (XIX) which, on recrystallization from Skellysolve B, was obtained in the form of small white needles melting at 117–118°.

Five grams of the above alcohol containing 6 drops of 3 *N* sulfuric acid was distilled to give the desired ethylene derivative in the form of a pale yellow, viscous oil, b.p. 200–205° (0.25 mm.).