CO_2H, 372-09-8; $(CH_3)_2CH(CH_2)_2Br$, 107-82-4; caffeine, 58-08-2; 8-phenylcaffeine, 6439-88-9; theophylline, 58-55-9; 8-methyltheophylline, 830-65-9; 8-phenyltheophylline, 961-45-5; 8-(*p*-hydroxyphenyl)theophylline, 85872-69-1; 8-(*m*-hydroxyphenyl)-theophylline, 85872-68-0; 8-(*o*-hydroxyphenyl)theophylline, 85872-57-7; 8-(*p*-aminophenyl)theophylline, 85872-66-8; 8-(*m*-aminophenyl)theophylline, 85872-65-7; 8-(*o*-aminophenyl)theophylline, 18830-58-5; 8-(*p*-carboxyphenyl)theophylline, 85872-58-8; 8-(*m*-carboxyphenyl)theophylline, 85872-52-2; 8-(*o*-carboxyphenyl)theophylline, 78164-01-9; 8-(*p*-sulfophenyl)theophylline, 80206-91-3; 1,3-dipropylxanthine, 31542-62-8; 1,3-dipropyl-8-phenylxanthine, 85872-53-3; 1,3-dipropyl-8-(2-amino-4-chlorophenyl)xanthine, 85872-51-1; 1,3-dipropyl-8-(*p*-hydroxyphenyl)-xanthine, 94781-76-7; 1,3-dipropyl-8-(*p*-carboxyphenyl)xanthine, 94781-78-9; 1,3-dipropyl-8-(*p*-sulfophenyl)xanthine, 89073-57-4; 1-isoamyl-3-isobutyl xanthine, 63908-26-9; 1-isoamyl-3-isobutyl-8-phenylxanthine, 94781-84-7; 1-isoamyl-3-isobutyl-8-(*p*-sulfophenyl)xanthine, 94781-85-8; 5,6-diamino-1,3-dimethyluracil, 5440-00-6; 6-amino-1,3-dimethyl-5-[(*p*-hydroxybenzylidene)-amino]uracil, 94781-72-3; 6-amino-1,3-dimethyl-5-[(*p*-nitrobenzylidene)amino]uracil, 76473-17-1; 8-(*p*-nitrophenyl)theophylline, 1094-63-9; 6-amino-1,3-dimethyl-5-[(*p*-carboxybenzylidene)amino]uracil, 94781-73-4; 1,3-dimethyl-6-amino-5-(*p*-sulfobenzamido)uracil, 94781-74-5; 1,3-dipropyl-6-aminouracil, 41862-14-0; 1,3-dipropyl-6-amino-5-nitrosouracil, 81250-33-1; 5,6-diamino-1,3-dipropyluracil, 81250-34-2; 6-amino-5-benzamido-1,3-dipropyluracil, 94781-75-6; 6-amino-5-[(*p*-carboxybenzylidene)amino]-1,3-dipropyluracil, 94781-77-8; 1,3-dipropyl-6-amino-5-(*p*-sulfobenzamido)uracil, 94781-79-0; 1-isobutyl-6-aminouracil, 56075-75-3; 1-isobutyl-3-isoamyl-6-aminouracil, 94781-80-3; isoamyl tosylate, 2431-75-6; 1-isobutyl-3-isoamyl-5-nitroso-6-aminouracil, 94781-81-4; 1-isobutyl-3-isoamyl-5,6-diaminouracil, 94781-82-5; 1-isobutyl-3-isoamyl-5-formamido-6-aminouracil, 94781-83-6; adenosine, 58-61-7.

Structure–Activity Relationship of Estrogens: Receptor Affinity and Estrogen Antagonist Activity of Certain (*E*)- and (*Z*)-1,2,3-Triaryl-2-propen-1-ones[1,2]

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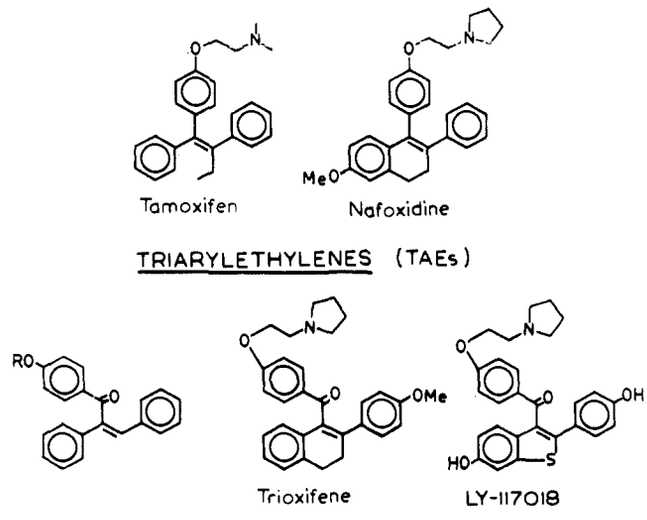
(*E*)- and (*Z*)-1,2,3-triphenyl-2-propen-1-ones and some of their phenolic and alkoxy analogues, substituted at the para position in one or more of the aromatic rings, were synthesized and assigned geometry on the basis of their spectroscopic data. The structure–activity relationship of the triarylpropenones was studied from the point of view of their estrogen receptor affinity and estrogen agonist and antagonist activities. (*E*)- as well as (*Z*)-propenones were found to compete with estradiol for binding with the mouse uterine cytosol receptors, with phenolic analogues usually more potent than the unsubstituted as well as alkoxypropenones. The (*E*)-propenones, which have now emerged as a new group of estrogen receptor ligands, were found to differ from *Z* isomers quite markedly in their binding specificities. The uterotrophic and antiuterotrophic assays in immature mice revealed that while some of the compounds were marginally estrogenic, nearly all the isomeric propenones were antiestrogenic to a varying degree.

The success of antiestrogen therapy in the treatment of certain hormone-responsive breast cancers[3,4] has led to a resurgence of interest in the molecules which can prevent estrogens from exercising their full biochemical effects. These interests relate both to the design of more effective antiestrogens and to elucidation of the mechanism of action of those already known. The majority of the present-day antiestrogens, such as, tamoxifen [(*Z*)-2-[4-(1,2-diphenyl-1-butenyl)phenoxy]-*N*,*N*-dimethylethanamine] and nafoxidine [1-[2-[4-(3,4-dihydro-6-methoxy-2-phenyl-1-naphthalenyl)phenoxy]ethyl]pyrrolidine], belong to a common chemical category, the triarylethylenes (TAEs) (see Chart I). The biological profile of the TAEs is characterized by mixed agonist–antagonist action, and their antagonist activity depends rather crucially on certain favorable circumstances of molecular geometry, substitution pattern, and even on the animal species employed for investigation.[5–11] While some of these features of TAE action are attracting considerable attention, the need for more effective antiestrogens continues to be an important one.

Chart I



Certain (*Z*)-1,2,3-triaryl-2-propen-1-ones (*Z*-TAPs) (see Chart I) have recently emerged as a new group of anti-

(1) CDRI Communication No. 3326.
(2) Dedicated to Dr. Nitya Anand on his 60th birthday.
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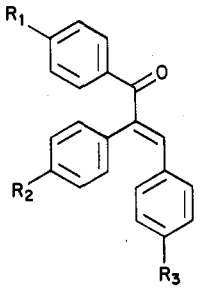
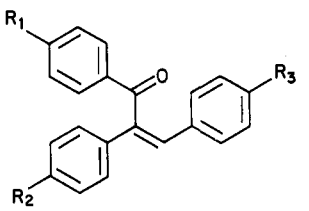
Table I. Physical Data of the (*E*)- and (*Z*)-1,2,3-Triaryl-2-propen-1-ones



E



Z

no.	R_1	R_2	R_3	geom[a]	solv[b]	mp, °C	TLC[c]	IR ν CO, cm^{-1}	formula	anal. or ref
1a	H	H	H	*E*	A	96.5–97.5	A	1640	$C_{21}H_{16}O$	23, 24
1b	H	H	H	*Z*	B	82.5–83.5	A	1665	$C_{21}H_{16}O$	23, 24
2a	OMe	H	H	*E*	B	80.0–80.5	A	1640	$C_{22}H_{18}O_2$	26
2b	OMe	H	H	*Z*	B	78–79	A	1655	$C_{22}H_{18}O_2$	26
3a	OH	H	H	*E**	C	187.0–187.5	B	1640	$C_{21}H_{16}O_2$	C, H
3b	OH	H	H	*Z**	C	70–71	B	1655	$C_{21}H_{16}O_2$	C, H
4a	OPy[d]	H	H	*E*		oil	C	1645		
4b	OPy	H	H	*Z*		oil	C	1665		
5a	H	OMe	H	*E*	A	85.0–85.5	C	1645	$C_{22}H_{18}O_2$	C, H
5b	H	OMe	H	*Z*	A	120–121	C	1665	$C_{22}H_{18}O_2$	C, H
6	H	OH	H		D	165.0–165.5	D	1625	$C_{21}H_{16}O_2$	C, H
7a	H	OAc	H	*E*	A	111	D	1640	$C_{23}H_{18}O_2$	C, H
7b	H	OAc	H	*Z*	A	150.0–150.5	D	1665	$C_{23}H_{18}O_2$	C, H
8a	H	H	OMe	*E*	C	80–81	C	1645	$C_{22}H_{18}O_2$	C, H
8b	H	H	OMe	*Z*	C	110.5–111.5	C	1655	$C_{22}H_{18}O_2$	C, H
9	H	H	OH		C	178.0–178.5	C	1615	$C_{21}H_{16}O_2$	C, H
10	H	H	OAc		A	122.5–123.5	D	1655	$C_{23}H_{18}O_3$	C, H
11a	OH	OH	H	*E**	C	115.0–115.5	E	1580	$C_{21}H_{16}O_3$	C, H
11b	OH	OH	H	*Z**	E	210.0–210.5	E	1605	$C_{21}H_{16}O_3$	C, H
12a	OH	H	OH	*E**		NC[e]	F	1600		
12b	OH	H	OH	*Z**	F	186.0–186.5	F	1625	$C_{21}N_{16}O_3$	C, H
13	OAc	H	OAc		F	122.0–122.5	G	1667	$C_{22}H_{20}O_5$	C, H
14a	H	OH	OH	*E**	E	206.5–207.5	H	1600	$C_{21}H_{16}O_3$	C, H
14b	H	OH	OH	*Z**	A	100–101	H	1655	$C_{21}H_{16}O_3$	C, H
15	OH	OH	OH		G	167–170	I	1605	$C_{21}H_{16}O_4$	C, H

[a] Those marked with an asterisk are tentative. [b] Solvents of crystallization are as follows: A = benzene–hexane, B = hexane, C = benzene, D = ethyl acetate–hexane, E = benzene–heptane, F = chloroform–hexane, G = ethyl acetate–benzene. [c] TLC solvent systems with which the *E* and *Z* isomers give different R_f values; A = benzene–hexane (1:4), B = ethyl acetate–benzene (1:10), C = benzene, D = ethyl acetate–benzene (1:20), E = methanol–chloroform (1:15), F = ethyl acetate–benzene (1:4), G = ethyl acetate–benzene (1:6), H = ethyl acetate–chloroform (1:7), and I = ethyl acetate–benzene (1:1). All compounds are on silica gel plates except for **4a** and **4b**, which are on basic alumina plates. [d] Py = pyrrolidinoethyl. [e] NC = noncrystallizable.

estrogens. Certain acyclic TAPs, substituted with a pyrrolidinoethoxy residue at the para position in the aroyl moiety, were first shown in this institute to act as postcoital contraceptives in rodents, with *Z* isomers more potent than the *E*-TAPs.[12–14] More recently, trioxifene [[3,4-dihydro-2-(4-methoxyphenyl)-1-naphthalenyl][4-[2-(1-pyrrolidinyl)ethoxy]phenyl]methanone] and LY-117018-[[6-hydroxy-2-(4-hydroxyphenyl)benzo[*b*]thien-3-yl][4-[2-(4-pyrrolidinyl)ethoxy]phenyl]methanone] [[6-hydroxy-2-(4-hydroxyphenyl)benzo[*b*]thien-3yl][4-[2-(1-pyrrolidinyl)ethoxy]phenyl]methanone] (Chart I), which incorporate the *Z*-TAP moiety in a rigid molecular framework, have emerged as some of the most potent antiestrogens yet known.[15–17] Since the chemical structure of *Z*-TAPs differs from that of the TAEs in an important respect, it was of interest to study the structure–activity relationship (SAR) of these molecular types, particularly from the point of view of their receptor affinity and estrogen antagonist activities. While receptor binding specificity of the TAEs has been studied extensively,[10,18–22] similar information on the *Z*-TAPs is lacking. We thus chose to synthesize certain acyclic TAPs and to investigate their receptor affinity and estrogen agonist and antagonist activities. The choice of acyclic analogues, which can exist as *E* and *Z* isomers, appeared particularly fruitful for an investigation of the geometrical specificity of this series of compounds. This was of interest because of the well-known fact that certain *E*- and *Z*-TAEs possess marked difference in their receptor affinities as well as in agonist and antagonist profiles.[9,10]

Chemistry

Synthesis. The *E*- and *Z*-TAPs, listed in Table I, were synthesized via Aldol condensation between appropriately substituted 2-phenylacetophenones and benzaldehydes,

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followed in some cases by further chemical elaborations. During Aldol condensation the products were obtained in excellent yield as a mixture of *E* and *Z* isomers, though in certain instances only one isomer could be detected or isolated in pure form. The *E* isomers largely predominated among the unsubstituted and monosubstituted TAPs, while the reverse appeared to be true among di- and trisubstituted compounds.

The pyrrolidinoethyl ethers **4a** and **4b** were prepared individually via alkylation of the phenols **3a** and **3b** and were characterized by ^{1}H NMR and IR spectroscopy. Preparation of the pyrrolidinoethyl ether corresponding to the phenol **9** proved abortive, while the ether corresponding to the phenol **6** was chemically unstable and could not be purified to homogeneity.

The acetates **7a** and **7b** were prepared directly by condensation between 2-(4-acetoxyphenyl)acetophenone and benzaldehyde. Mild alkaline hydrolysis of **7a** furnished the isomerically pure phenol **6**. The phenol **9** was obtained as the sole product from the reaction between 2-phenylacetophenone and 4-hydroxybenzaldehyde. Acetylation of **9** furnished the compound **10** as the sole characterizable product. The phenol **12b** was obtained as the major compound from Aldol reaction while its isomer **12a**, purified by PLC, was prone to isomerization and was noncrystallizable. The diacetate **13** was the only well characterizable product from acetylation of the mixture of phenols **12a** and **12b**. The triphenol **15** was the only isomer obtained from reaction between 4′-hydroxy-2-(4-hydroxyphenyl)acetophenone and 4-hydroxybenzaldehyde.

Geometrical Assignments. Geometrical assignments of the TAP isomers were carried out essentially on the basis of their IR data. It is quite well established that there is steric inhibition of the enone resonance in *Z*-TAPs,[23,24] which results in the appearance of their carbonyl absorption bands at relatively higher wavenumbers than those in the *E* isomers.[24] The carbonyl absorption band positions of the TAP isomers are shown in Table I. Those absorbing at relatively lower wavenumbers (those marked *a*), have uniformly been assigned *E* geometry. The assignments of all the phenolic TAPs are, however, uncertain and must be considered as tentative for the present. This is so because of the possibility of intermolecular hydrogen bonding in these molecules and its complex and variable influence on the position of their carbonyl absorption bands. Solution-phase IR was of little help in this respect because of the poor solubilities of the polyphenols in appropriate nonpolar solvents and their ready reequilibration in polar solvents.

The unsubstituted, methoxy, and monophenolic TAPs were reasonably stable provided their solutions were protected from excessive exposure to light. The geometrical identities of **1a** and **1b** and that of their methoxy analogues were further substantiated by ^{13}C NMR spectra. According to these data, to be published elsewhere in detail,[25] C-3 of the propenone moiety was found always to absorb several ppm downfield in *E*-TAPs as compared to that in *Z* isomers. This marked geometrical dependence of the chemical shift of C-3 obviously originates from the relatively higher electron density at this carbon center in *Z*-TAPs and thus substantiates the lack of enone resonance in these isomers.

Biological Results

Receptor Affinities. Receptor affinity studies were carried out with the mouse uterine cytosol at 4 °C as previously described.[26] The comparative ability of the parent and the monosubstituted TAPs to inhibit the receptor binding of [^{3}H]E_2 was studied first at a single 1×10^{-4} M competitor concentration, against a 2×10^{-9} M concentration of the radioligand. All the compounds thus studied were found to compete with E_2 for receptor binding, though with widely variable efficiencies. Compounds **1b**, **2a**, **2b**, **3b**, **5b**, and **8b** were the least effective, causing less than 50% inhibition in the radioligand binding. These were excluded from the concentration-dependent binding inhibition assay which was next performed with all the other TAPs (except for those found unstable) together with that of E_2. The plots of binding inhibition vs. log competitor concentration for all the TAPs were constructured and found nearly parallel to that of the E_2. The relative binding affinity (RBA) values were computed from these data as the ratio of the molar concentration of E_2 and the TAPs required to decrease [^{3}H]E_2 binding by 50%, times 100. The RBA values are shown in Table II. For the sake of comparison, RBA values of compounds which caused less than 50% binding inhibition at 1×10^{-4} M concentration are shown as <0.001 in this table.

From the RBA data it is evident that the *E* as well as *Z*-TAPs possess receptor affinities, with phenolic analogues generally more potent than the others. While no compound approaches E_2 in its affinity, some of the phenols are reasonably potent. Marked difference in the affinity of some of the *E*- and *Z*-TAPs is noticed, pointing to a difference in their binding specificities. At least as judged from the data of the unsubstituted and certain monosubstituted compounds possessing well-defined geometries, *E*-TAPs are seen to be consistently more potent than the *Z* isomers. This conclusion is inescapable even if allowance is made for the possibility of some isomerization during RBA experiments. In the unsubstituted and monosubstituted series, the *E*-TAPs are thermodynamically much more stable, and a certain extent of isomerization can only lead to an underestimation of their RBA values, while just the reverse would be expected to happen among *Z*-TAPs.

All the phenolic TAPs, except for **3b**, are found to interact with the receptor with nearly comparable affinities, and among these **3a** and **11b** are clearly the most potent. A general correspondence in the affinity of the phenols and their acetate derivatives is noticed, which may perhaps result from hydrolysis of the acetoxyl functions during the binding assays.

Though their geometric identities are uncertain, it is clear that while incorporation of the pyrrolidinoethyl chain causes an increase in the receptor affinity of one of the phenols, it has a detrimental effect on the binding of the other. This appears to be a further indication of the fundamental difference in the receptor binding specificity of the *E*- and *Z*-TAPs.

Estrogenic and Antiestrogenic Activities. Immature rat and mouse uterotrophic and antiuterotrophic assays were employed to study the estrogenic and antiestrogenic activities of the TAPs. An experiment was first performed in mice with a single 100-μg dose of each compound. The results from this study are compiled in Table II. From these data it is noticed that except for **3a** and **13**, which are marginally active, every other compound is devoid of uterotrophic activity at this comparatively high dose. The uterotrophic activities of **3a** and **13** were also assayed at

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Table II. Receptor Affinity and Biological Activity Data of the (*E*)- and (*Z*)-1,2,3-Triaryl-2-propen-1-ones in Mice

no.	RBA[a]	uterine wt (mg; mean ± SD)[b]		antiuterotrophic % inhibn[c]
		without E_2	with concomitant E_2	
1a	0.012	4.4 ± 0.7 (8)	15.4 ± 4.1 (7)	51
1b	<0.001	3.0 ± 0.8 (6)	15.3 ± 3.5 (6)	51
2a	<0.001	5.0 ± 2.7 (6)	20.2 ± 5.4 (6)	27
2b	<0.001	4.8 ± 0.9 (6)	16.0 ± 4.7 (6)	48
3a	2.02	9.7 ± 2.0 (8)	18.5 ± 5.0 (6)	36
3b	<0.001	3.2 ± 1.3 (6)	13.6 ± 3.9 (6)	60
4a	0.025			
4b	0.002			
5a	0.02	4.7 ± 0.7 (6)	18.5 ± 2.4 (6)	36
5b	<0.001	3.5 ± 1.2 (6)	15.3 ± 2.1 (5)	52
6	0.28	2.9 ± 1.8 (6)	18.4 ± 3.6 (6)	36
7a	0.28	4.2 ± 1.8 (6)	21.5 ± 0.6 (6)	20
7b	0.24	4.5 ± 1.3 (6)	17.8 ± 3.3 (6)	39
8a	0.09	4.3 ± 1.3 (6)	17.3 ± 2.9 (6)	42
8b	<0.001	4.6 ± 1.2 (5)	15.3 ± 3.5 (6)	52
9	0.22	5.0 ± 1.3 (6)	14.0 ± 4.3 (6)	58
10	0.42	5.9 ± 1.2 (6)	17.3 ± 3.1 (6)	42
11a	0.37	6.8 ± 1.8 (5)	21.9 ± 4.1 (6)	18
11b	4.75	4.1 ± 0.7 (8)	14.7 ± 2.2 (8)	55
12b	0.40	3.4 ± 0.7 (5)	22.3 ± 4.4 (6)	16
13	1.02	14.0 ± 1.8 (8)	14.0 ± 1.5 (5)	58
14a	0.57	5.5 ± 1.0 (6)	16.9 ± 2.5 (6)	44
14b	0.48	5.6 ± 2.3 (6)	16.5 ± 2.3 (6)	47
15	0.55	4.4 ± 1.0 (8)	12.0 ± 2.0 (6)	69
E_2	100	25.5 ± 2.8 (25)		
control		3.9 ± 1.0 (10)		

[a] The values represent the mean from two independent determinations in each case. [b] One hundred micrograms/animal of each compound was administered. For antiuterotrophic assay, 1 μg/animal of E_2 was coadministered. The number of animals in each case is shown in the parentheses. [c] Computed as $100 \times [(W_{ET} - W_C)/(W_E - W_C)]$, wherein W_{ET}, W_E, and W_C refer to the mean uterine weights from animals treated with the TAPs, E_2, and the vehicle alone, respectively.

Table III. Uterotrophic and Antiuterotrophic Activity Data of the Triphenol **15** in Rats

dose, μg/rat	uterine weight,[a] mg	
	without E_2	with concomitant E_2[b]
0.5		55.3 ± 3.2
5.0	20.5 ± 2.5	61.2 ± 5.0
50	18.4 ± 3.4	52.0 ± 5.6
100	19.5 ± 1.7	
500	26.5 ± 1.0	53.6 ± 7.0
1000	25.2 ± 2.0	40.1 ± 2.9
control	17.6 ± 4.6	60.6 ± 9.6

[a] Each value is mean from at least six animals ± SD. [b] One microgram/animal of E_2 was coadministered.

lower doses (data not shown) and both these were found to be inactive at doses lower than 50 μg.

From the antiuterotrophic activity data in mice (Table II), all the TAPs tested, irrespective of their geometry or substitution pattern, are seen to be antiestrogenic, though their relative potencies vary somewhat. No discernible structure–activity trends are, however, noticed. The triphenol **15** appears to be the most potent antiestrogen, causing nearly 70% decrease in E_2 stimulated uterine weight (see comparative antiestrogenicity figures, Table II). However, many other compounds appear to have potencies closely approaching that of **15** and in certain instances may have potencies not significantly different from it. Antiuterotrophic activities of **1a**, **3a**, **11b**, and **13** were assayed at lower doses (data not shown), and no significant difference was noticed in their potencies down to a 5-μg dose.

A dose-dependent study was next undertaken with **15** along with that of tamoxifen as the reference compound. From these data, shown in Figure 1, compound **15** is seen to be inactive as an estrogen up to a 100-μg dose and marginally active at higher doses, while it manifests antiestrogenic activity of almost comparable magnitude in the entire dose range. Compared with this, tamoxifen

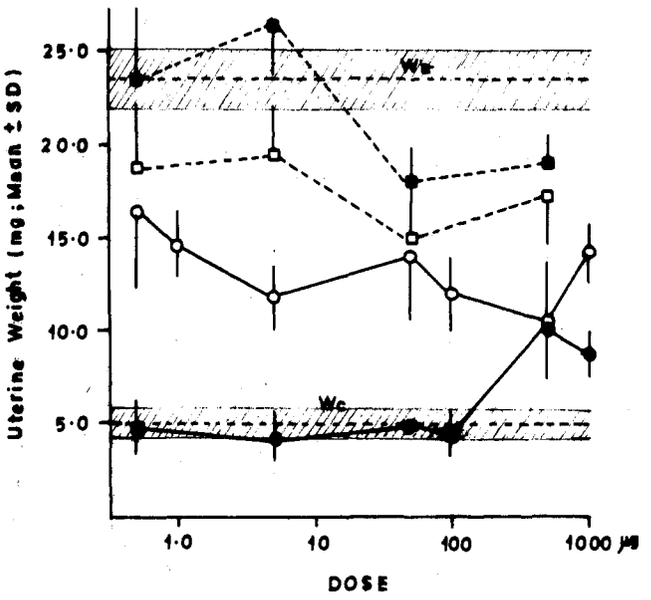


Figure 1. Uterotrophic (closed symbols) and antiuterotrophic (open symbols) activities of the triphenol **15** (○, ●) and that of tamoxifen (□, ■) in immature mice. Each value is the mean uterine weight ± SD from at least six animals. W_E and W_C refer to the mean uterine weights from animals treated with E_2 (1 μg) and the vehicle alone, respectively, with the shaded area representing the SD in each case.

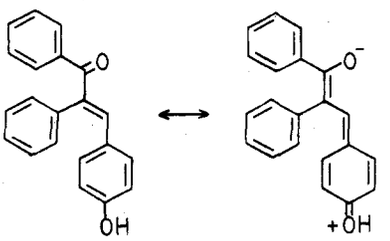
possesses the full measure of estrogenic activity. In the higher dose range, however, a certain degree of autoinhibition of the uterotrophic response is noticed with this compound, which is apparantly manifested in the antiuterotrophic assay as well.

Uterotrophic and antiuterotrophic activities of **15** were also assessed in rats, and the data are shown in Table III. The compound is found to be marginally active in this animal species, both as an estrogen and antiestrogen, but

only at comparatively high doses.

Discussion

Elucidation of the SAR of TAPs, the primary objective of this investigation, has suffered due to the propensity of the acyclic analogues to isomerize and from our inability to make unambiguous geometrical assignments in every case. Unlike TAEs, the acyclic TAPs suffer from the presence in these molecules of the geometrically labile and chemically susceptible 1,2-enone system, and the difficulties encountered in their handling are, therefore, hardly surprising. The tendency of some of the compounds, particularly phenols, to exist as single isomers or to reequilibrate into mixtures is apparently influenced, among other things, by the resonance isomerism of the kind shown below. These problems notwithstanding the findings reported here do permit certain valuable conclusions.



In light of their analogy with the trioxifen and LY-117018 structure, it was only natural to expect that the *Z*-TAPs would interact with the receptor. The receptor binding of *E*-TAPs has, however, come as a surprise, more so because these do not incorporate the *trans*-stilbene core which *Z* isomers share with the TAEs and is commonly presumed to be crucial for their accommodation in the E_2 site. The *E*-TAPs would thus appear to have emerged as a new group of receptor ligands which do not fit into our current conception of the estrogen binding site.[20]

It is evident from a vast body of literature[27] that, irrespective of their geometry, the simple TAEs, carrying at best hydroxy or methoxy substituents at para positions in their aromatic rings, are essentially estrogen agonists devoid of antagonist activity. The strong to moderate antiuterotrophic activity of even the simplest of the TAPs in mice and at doses where no uterotrophic activity is apparently manifested is indicative of a fundamental difference in the biological profile of the TAEs and the TAPs. The comparative activity of the triphenol **15** and that of tamoxifen in mice is particularly noteworthy in this context, suggestive as it is of a behavior of the TAPs which is quite contrary to that of the TAE antiestrogens in this animal species.[7] In rats the compound **15** acts as an estrogen agonist and antagonist, but at comparatively high doses, while showing no dissociation between these activities. This reveals that the biological activity of this compound varies quite markedly in rats and mice. Interestingly enough, this trend in species variation appears to be contrary to what is well-known for some of the TAE antiestrogens, viz., strong antiuterotrophic activity in rats and virtual absence of this activity in mice.[27]

In view of their chemical instability, we have not assessed the biological activities of the basic ethers **4a** and **4b**. We do not, therefore, know as yet whether the provision of the side chain would bring about any modulation in the activity of the acyclic TAPs, especially in relation to their geometrical isomerism. This is an important point for future investigation because it has been shown recently that the side chain is quite crucial for high receptor affinity as well as estrogen agonist and antagonist activities of trioxifene,[28] a *Z*-TAP.

To conclude, the findings reported here reveal that the acyclic TAPs compete with E_2 for receptor binding in vitro and act as estrogen agonists plus antagonists in the rodent uterotrophic asssays. The potent antiuterotrophic activity shown by the compounds in mice and the dissociation between their uterotrophic and antiuterotrophic effects observed in this animal species is particularly noteworthy as is the emergence of the *E*-TAPs as a new group of estrogen receptor ligands.

Experimental Section

General Procedures. The melting points were determined on a Townson Mercer apparatus and are uncorrected. The ^1H NMR spectra were recorded on Varian CFT-20, EM-360L, and Perkin-Elmer R-32 instruments, using tetramethylsilane as the internal reference. The mass spectral measurements were performed on a JEOL JMS-D300 instrument fitted with a direct inlet system. The IR spectra were recorded as KBr wafers or as neat films on Perkin-Elmer 157 or 557 infracord instruments. Thin-layer chromatography (TLC) was performed on silica gel or alumina plates.

Synthetic Procedures. Various 1,2-diarylethanones were either obtained commercially or prepared according to the reported procedures.

2-[4-(Acetyloxy)phenyl]-1-phenylethanone. This was prepared by routine acetylation of the corresponding hydroxyphenylethanone,[13] with acetic anhydride–pyridine. The compound was crystallized from ethyl acetate–hexane: mp 140 °C; IR (KBr) 1680 (Ar CO), 1745 ($OCOCH_3$) cm^{-1}; ^1H NMR τ 1.75–2.15 (2, m, Ar H, ortho to CO), 2.35–3.05 (7, m, Ar H), 5.70 (2, s, CH_2CO), 7.70 (3, s, $OCOCH_3$). Anal. Calcd for $C_{16}H_{14}O_3$: C, 75.60; H, 5.51. Found: C, 75.80; H, 5.40.

(*E*)- and (*Z*)-1,2,3-Triaryl-2-propen-1-ones (TAPs): General Procedure. Equimolar proportions of appropriate 1,2-diarylethanones and arylaldehydes dissolved in anhydrous benzene or in anhydrous THF–benzene (1:9) (usually 100 mL for each 50 mmol of the reactants) were refluxed in a Dean–Stark assembly, in the presence of glacial AcOH and anhydrous piperidine (2.8 and 1.0 mL, respectively, for each 50 mmol of the reactants), for 12–36 h. During this period, the solvent in the Dean–Stark assembly was replaced several times with anhydrous solvent. After the completion of the reaction, the mixtures were cooled and mixed with equal proportions of water and in certain instances with ethyl acetate to achieve complete recovery of the products. The organic layers were separated, washed twice with equal volumes of water, dried (anhydrous Na_2SO_4), and concentrated in vacuo. The crude reaction products, obtained invariably in better than 90% yield, were then purified by chromatographic procedures or often straightway by fractional crystallization to obtain the pure *E*- and *Z*-TAPs. The isomeric TAPs were always found to be well resolved on TLC in the solvent systems indicated in Table I.

The physical data of **1a**, **1b**, **2a**, **2b**, **3a**, **3b**, **5a**, **5b**, **7a**, **7b**, **8a**, **8b**, **9**, **11a**, **11b**, **12a**, **12b**, **14a**, **14b**, and **15**, thus prepared, are shown in Table I, along with those of the others. The ^1H NMR and mass spectral data, which were, in general, of no help in making the geometrical assignments, are provided as supplementary material.

(*E*)- and (*Z*)-1-[4-[2-(1-Pyrrolidinyl)ethoxy]phenyl]-2,3-diphenyl-2-propen-1-ones (4a and 4b). These were prepared individually with phenols **3a** and **3b** as the starting material as follows. A mixture of the respective phenols (150 mg) with β-pyrrolidinoethyl chloride hydrochloride (250 mg), anhydrous acetone (4 mL), and anhydrous K_2CO_3 (1 g) were refluxed overnight, then cooled, and filtered. The filterates were concentrated in vacuo and subjected to chromatography over neutral alumina columns to obtain **4a** and **4b**, respectively, as oils.

2-(4-Hydroxyphenyl)-1,3-diphenyl-2-propen-1-one (6). This was obtained via hydrolysis of the acetate **7a** with 5% ethanolic

(27) For a recent review, see: Jordan, V. C.; Clark, E. R.; Allen, K. E. In "Non-Steroidal Antiestrogens"; Jordan, V. C., Sytherland, R. L., Ed.; Academic Press: Sydney, Australia, 1981; pp 31.

(28) Jordan, V. C.; Gosden, B. *Mol. Cell. Endocrinol.* **1982**, *27*, 291.

KOH at room temperature for 4 h. Usual workup of the reaction and crystallization of the product furnished **6** almost quantitatively.

3-[4-(Acetyloxy)phenyl]-1,2-diphenyl-2-propen-1-one (10). This was prepared by acetylation of the phenol **9** wth acetic anhydride in the presence of excess of pyridine. Usual workup of reaction and crystallization of the product furnished **10**.

1,3-Bis[4-(acetyloxy)phenyl]-2-phenyl-2-propen-1-one (13). This was prepared by acetylation of the mixture of phenols **12a** and **12b** with acetic anhydride in the presence of pyridine, followed by crystallization of the reaction product.

Biology. Materials. [2,3,6,7-^{3}H]Estradiol ([^{3}H]E_2, 100 Ci mmol^{-1}) was purchased from New England Nuclear Corp. and was assessed as >95% radiochemically pure by use of a Panax radio TLC scanner. Unlabeled estradiol (E_2) was obtained from Steraloids Inc., activated charcoal, Norit A, from Sigma Chemicals, and Dextran T-70 from Pharmacia Fine Chemicals. All other chemicals and reagents were of analytical or scintillation grade.

Female mice (21–23-days old, 8–12 g of body weight) of Swiss strain and rats (21–23-days old, 25–40 g of body weight) of Charles Foster strain were obtained from the CDRI rodent colony. For receptor binding experiments the mice were primed subcutaneously with 1 μg of E_2 each, 24 h prior to use, to increase the yield of the receptor protein in their uteri.

Preparation and Handling of the Test Solutions. Owing to the susceptibility of the TAPs to isomerization, particularly on light exposure, their stock solutions in DMF–buffer (1:1, v/v) for RBA assays and in propylene glycol–saline (1:1, v/v) for the bioassays were prepared without undue warming and with minimal light exposure. The solutions were, as far as possible, kept refrigerated and protected from light during the course of their use. Under these conditions nearly all the compounds retained their geometrical identities as assessed by periodic TLC examination.

Receptor Binding Experiments. Receptor binding procedures were essentially the same as reported earlier.[26] Briefly, the competition experiments with uterine cytosols were performed at 4 °C, 18-h incubation, and with triplicate tubes for each competitor concentration. Each incubate (260 μL) in TEA buffer (Tris-HCl, 10 mmol; EDTA, 1.65 mmol; NaN_3, 0.02%; pH 7.4) was 0.4 equiv in uteri, 7% in DMF, 2×10^{-9} M in [^{3}H]E_2, and $\sim 10^{-4}$ to 10^{-9} in the competitors. For the separation of free from bound [^{3}H]E_2, each incubate was treated at 4 °C for 15 min with a 100-μL aliquot of charcoal–dextran slurry (2.5% and 0.25%, w/v, respectively) in TEA buffer. Radioactivity was measured in a Packard Tricarb liquid scintillation spectrometer in 10 mL of methanol–toluene–dioxane mixture (1.5:2.5:2.5, v/v), containing 0.5% PPO, 0.01% POPOP, and 9% naphthalene.

Each experiment was performed in duplicate and the RBA values were always within 10% of each other.

Bioassays. For uterotrophic assay, various doses of the test compounds in 0.1 mL of propylene glycol–0.9% saline (1:1, v/v) were injected subcutaneously to the animals in groups of 6–10 on three consecutive days, while the control group received similar injections of the vehicle alone. The animals were autopsied 24 h after the last injection, and their uterine weights were recorded.

Antiuterotrophic assays were performed similarly, but in this case the animals were coadministered 1 μg of E_2 each in the same vehicle but at a different site. The control group in this case received 1 μg of E_2 plus the vehicle alone, also at two different sites.

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Registry No. **1a**, 7474-65-9; **1b**, 7512-67-6; **2a**, 94348-26-2; **2b**, 94348-27-3; **3a**, 63645-39-6; **3b**, 94348-28-4; **4a**, 94348-29-5; **4b**, 94348-30-8; **5a**, 94348-31-9; **5b**, 94348-32-0; **6**, 94369-84-3; **7a**, 94369-85-4; **7b**, 94369-86-5; **8a**, 34236-65-2; **8b**, 34236-57-2; **9**, 94348-22-8; **10**, 94348-23-9; **11a**, 94348-33-1; **11b**, 94348-34-2; **12a**, 94348-35-3; **12b**, 94348-36-4; **13**, 94348-24-0; **14a**, 94348-37-5; **14b**, 94348-38-6; **15**, 94348-25-1; 2-[4-(acetyloxy)phenyl]-1-phenylethanone, 94348-39-7; 2-(4-hydroxyphenyl)-1-phenylethanone, 6420-90-2; β-pyrrolidinoethyl chloride hydrochloride, 7250-67-1.

Supplementary Material Available: ^{1}H NMR and mass spectral data of all the compounds (3 pages). Ordering information is given on any current masthead page.

5-Cinnamoyl-6-aminouracil Derivatives as Novel Anticancer Agents. Synthesis, Biological Evaluation, and Structure–Activity Relationships

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A biological evaluation in the series of 5-cinnamoyl-6-aminouracils has been undertaken. These compounds have been found to be in an extended planar conformation fitting well with a possible stacking interaction between the nucleic bases of DNA; thus an eventual anticancer activity by intercalation could be hoped. 1,3-Dimethyl-5-cinnamoyl-6-aminouracil was found to be active when administered ip against ip-implanted P388 leukemia in vivo (percent T/C = 124). Two other compounds, 1,3-dimethyl-5-cinnamoyl-6-[(2-morpholinoethyl)amino]uracil and 1,3-dimethyl-5-cinnamoyl-6-[(2-piperidinoethyl)amino]uracil, bearing a hydrophilic side chain on the 6-amino group, have exhibited cytoxic activity in vitro against L1210 leukemia. Structure–activity relationships have been determined from these results and from studies of biological interactions with DNA.

Uracil derivatives play an important role in the field of biology as fundamental constituents of nucleic acids. In medicinal chemistry, 1,3-dimethyl-6-aminouracil (**1**) is well-known as a starting compound for the synthesis of a number of xanthines related to theophyllin.[1] In our hands, from this molecule **1**, new condensed heterocycles have been synthetized, which often exhibited interesting pharmacological activity.[2–4] In the course of those studies, we have pointed out that 1,3-dimethyl-6-aminouracil derivatives substituted at the 5-position by a carbonyl group were conformationally stabilized by a strong hydrogen bond between the CO group and one of the protons of the 6-amino groups.[5] The latter concept led us to the design

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