Experimental Section[13]

11β-Hydroxy-4-methylestra-1,3,5(10)-trien-17-one (IIa).[5]—To a mixture of 3.0 g of $LiAlH_4$ and 250 ml of anhydrous Et_2O, stirred and heated under reflux, was added a mixture of 2.0 g of 11β-hydroxyandrosta-1,4-diene-3,17-dione 17-ethylene ketal (I)[14] in 25 ml of THF. After addition was complete, the addition funnel was rinsed with 10 ml of THF, and the rinse was added to the reaction mixture. The resultant mixture was stirred and heated under reflux for 16 hr. Then it was cooled in an ice bath and successively treated with 20 ml of Me_2CO, 50 ml of H_2O, and 150 ml of 6 *N* HCl. The reaction mixture was distilled under reduced pressure with a minimum of heating to remove the ether. The residue was stirred at room temperature for 1.75 hr. Then it was extracted ($CHCl_3$), and the extract was washed (H_2O), dried (Na_2SO_4), and concentrated to a small volume by distillation under reduced pressure. The residue was diluted with hexane and cooled to 0° to afford 0.78 g of IIa: mp 237.5–243°; λ_{max}^{MeOH} 263–264 mμ (ε 313); λ^{KBr} 2.83, 5.76, 6.29, 13.35 μ. After crystallization from $CHCl_3$–hexane, IIa melted at 235.5–244.5°, $[\alpha]^{27}D$ +204.5° (*c* 1, $CHCl_3$). 11β-Hydroxy-4-methylestra-1,3,5(10)-trien-17-one (IIa) appeared to undergo a change in crystalline form just below its melting point, thus accounting for the broad melting point range [lit.[5] mp 244–246° with change in crystalline structure at 224–226°, $[\alpha]^{25}D$ +95° (dioxane)]. *Anal.* ($C_{19}H_{24}O_2$) C, H. Recrystallization of IIa from DMF raised the melting point to 259–260°, $[\alpha]^{27}D$ +208° (*c* 1, $CHCl_3$).

11β-Acetoxy-4-methylestra-1,3,5(10)-trien-17-one (IIb).—A mixture of 2.85 g of IIIa, 40 ml of pyridine, and 40 ml of Ac_2O was heated on the steam bath for 4 hr after which time it was poured into a mixture of ice and water. The mixture was neutralized with 6 *N* HCl. The resultant solid was collected, washed (H_2O), and dried, mp 197–206.5°. Crystallization from ether afforded 2.66 g of IIb: mp 206.5–208.5°; λ^{KBr} *ca.* 5.73, 6.29, 8.04, 13.45 μ; nmr, 421.5, 357 (quartet, J = 3 cps), 134.5, 110, 63 cps; $[\alpha]^{27}D$ +94° (*c* 1, $CHCl_3$). *Anal.* ($C_{21}H_{26}O_3$) C, H.

11-β-Acetoxy-17α-ethynyl-4-methylestra-1,3,5(10)-trien-17β-ol (IIId).—To a solution of 4.50 g of IIb in 150 ml of THF was added 10.00 g of the lithium acetylide–ethylenediamine complex.[14] While acetylene was bubbled in, the reaction mixture was stirred at room temperature for 16 hr. The reaction mixture was treated with 150 ml of H_2O and stirred at room temperature for an additional 1 hour. Then it was acidified with 6 *N* HCl. The acidified mixture was poured into a mixture of ice and water. The yellow gum was collected, washed (H_2O), and dissolved in $CHCl_3$. The solution was dried (Na_2SO_4) and evaporated to dryness to afford a viscous oil. A 4.42-g sample of the oil was chromatographed on 310 g of silica gel. The column was eluted initially with C_6H_6 and then with varying proportions of EtOAc and C_6H_6. Elution of the column with 5% EtOAc in C_6H_6 gave 0.94 g of IIId as a crystalline product. Crystallization from $CHCl_3$–hexane afforded 0.71 g of IIId: mp 201.5–209.5°; λ^{KBr} 2.83, 3.06, 4.72, 5.81, 6.30, 7.92, 7.98, 13.38 μ; $[\alpha]^{26}D$ −19.5° (*c* 1, $CHCl_3$). Another crystallization from the same solvents raised the melting point to 217–219°. Admixed with IIb, IIId melted at 193–199.5°. *Anal.* ($C_{23}H_{28}O_3$) C, H.

17α-Ethynyl-11β-hydroxy-4-methylestra-1,3,5(10)-trien-17β-ol (IIIc).—Continued elution of the aforementioned column with 10% EtOAc in C_6H_6 gave 1.33 g of IIIc as a crystalline substance. Crystallization from ether–hexane afforded 0.87 g of IIIc: mp 186.5–190.5°; λ^{KBr} 2.78, 2.84, 3.08, 4.77, 6.32, 13.37 μ. The melting point was raised to 192–195° on further crystallization from ether, $[\alpha]^{26}D$ +71.0° (*c* 1, $CHCl_3$). *Anal.* ($C_{21}H_{26}O_2$) C, H.

4,17α-Dimethylestra-1,3,5(10)-triene-11β,17β-diol (IVb).—To 40 ml of 3 *M* MeMgBr in Et_2O, stirred at room temperature, was added a solution of 1.10 g of 11β-acetoxy-4-methylestra-1,3,5(10)-trien-17β-one (IIb) in 15 ml of THF. After addition was complete, the addition funnel was rinsed with 20 ml of anhydrous Et_2O, and the rinse was added to the reaction mixture. The reaction mixture was stirred and heated under reflux for 4 hr. Then it was cooled in an ice bath. The reaction mixture was successively treated with H_2O, acidified with 6 *N* HCl, diluted (Et_2O), and shaken. The ether phase was separated, washed (H_2O), dried (Na_2SO_4), and distilled under reduced pressure until a solid appeared. The residue was cooled to 0°. The solid was collected, yield 0.60 g. It was crystallized from ether to afford 0.45 g of IVb: mp 141.5–143.5° with melting and resolidification below 120°; nmr, 421–435.5 (multiplet), 289 (quartet, J = 3 cps), 132.5, 75, 68.5 cps; λ^{KBr} 2.77, 2.88, 6.32, 13.33 μ; $[\alpha]^{24}D$ +120° (*c* 1, $CHCl_3$). *Anal.* ($C_{20}H_{28}O_2$) C, H.

17β-(2-Hydroxyacetyl)-4-methylestra-1,3,5(10)-trien-17α-ol (V).[10]—The procedure of Caspi, *et al.*, was modified. To a mixture of 5.0 g of $LiAlH_4$ and 500 ml of anhydrous Et_2O, stirred and heated under reflux, was added a mixture of 5.0 g of the bismethylenedioxy derivative of prednisolone in 150 ml of THF. After the addition was complete, the addition funnel was rinsed with 50 ml of THF, and the rinse was added to the reaction mixture. The mixture was stirred and heated under reflux for 44 hr, then it was chilled in an ice bath. It was successively and cautiously treated with 20 ml of Me_2CO, 200 ml of H_2O, and 200 ml of 12 *N* HCl. The reaction mixture was distilled under reduced pressure with gentle heating to remove the ether. The residue was stirred at room temperature for 3.5 hr and extracted with $CHCl_3$; the extract was washed (H_2O), dried (Na_2SO_4), and distilled under reduced pressure until a crystalline product appeared. The residue was diluted with hexane and cooled to 0° to afford 1.23 g of V: mp 194–201.5°; λ^{KBr} 2.89, 5.85, 6.31, 13.38 μ. Crystallization from $CHCl_3$–hexane and then from C_6H_6 afforded V as a pale yellow crystalline product: mp 214–217°; $[\alpha]^{24}D$ +118° (*c* 1, $CHCl_3$) [lit.[10] mp 191–193°, $[\alpha]^{27}D$ +112° ($CHCl_3$)]. *Anal.* ($C_{21}H_{28}O_4$) C, H.

(13) Melting points were taken on a Fisher-Johns melting block and are corrected. Nmr spectra were determined in deuteriochloroform on a Varian A-60 spectrometer, and the signals are reported downfield with respect to tetramethylsilane as an internal standard. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

(14) O. F. Beumal, Jr., and R. F. Harris, *J. Org. Chem.* **28**, 2775, (1963); **29**, 1872 (1964).

Drug Latentiation. The Preparation and Preliminary Pharmacological Evaluation of Some Mephenesin Aryloxyacetates

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Esterification of one or more free hydroxyl groups of a drug is one of the possible means of achieving "latentiation" of the drug itself, *i.e.*, transformation into a derivative from which the active compound is regenerated *in vivo*.[2] The activity of the latentiated drug will depend, among other things, (a) on the rate of absorption, distribution in the tissues, and accumulation on the target area; (b) on the rate of "bioactivation," *i.e.*, *in vivo* hydrolysis to liberate the parent compound.[3] In recent times, attempts have been made to apply some basic concepts of intramolecular catalysis to drug latentiation, by esterifying drugs with acids, whose esters are known to undergo facilitated hydrolysis.[4] On the same ground, other labile drug derivatives such as ethers and amides have been prepared.[5]

Our interest in this field arose from the observation that some aryloxyacetic acids, also known as plant growth regulators, have been found to confer upon esterified drugs enhanced intensity and duration of

(1) Names of authors are in alphabetical order. This work was supported by a grant from Consiglio Nazionale delle Ricerche.

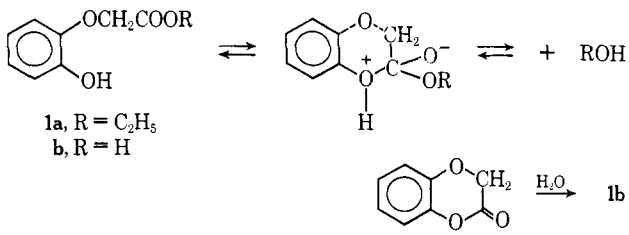
(2) (a) N. J. Harper, *J. Med. Pharm. Chem.*, **1**, 467 (1959); (b) N. J. Harper, *Progr. Drug Res.*, **4**, 241 (1962).

(3) E. J. Ariens, "Molecular Pharmacology," Vol. 1, Academic Press Inc., New York, N. Y., 1964, p 81.

(4) S. M. Kupchan, A. F. Casy, and J. V. Swintosky, *J. Pharm. Sci.*, **54**, 514 (1965).

(5) (a) S. M. Kupchan and A. F. Casey, *J. Med. Chem.*, **10**, 959 (1967); (b) S. M. Kupchan and A. C. Isenburg, *ibid.*, **10**, 960 (1967).

action.[6] We had previously reported that ethyl *o*-hydroxyphenoxyacetate (**1a**) undergoes a facilitation of hydrolysis through intramolecular catalysis, the lactone **2** being a probable intermediate in the process.[7] It was presumed that esterification of a drug with *o*-hydroxyphenoxyacetic acid (**1b**) would give a labile derivative, whose rapid breakdown *in vivo* might reason-



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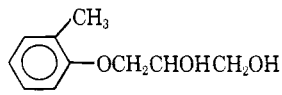
ably be expected. One of the hydrolysis products, **1b**, is relatively nontoxic and had been used in the past, as the calcium or sodium salt, as an antipyretic.[8] We felt it would be of interest to compare the intensity and duration of action of a drug derivative of **1b** with respect to other aryloxyacetates of the same drug, in order to gain some basic knowledge on the "latentiating" capacity and mechanism of these ester derivatives. A comparison between aryloxyacetates and other esters might also be useful.

The muscle relaxant 3-*o*-toloxy-1,2-propanediol (me-



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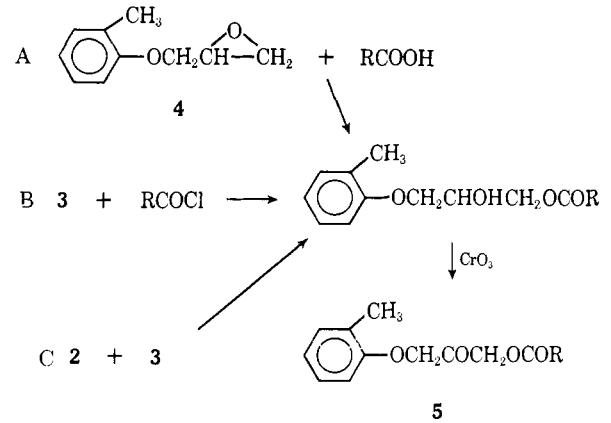
phenesin, **3**) was chosen as the model drug for this study. The compound is characterized by an extremely short duration of action, due to its rapid *in vivo* oxidation to β-(*o*-toloxy)lactic acid.[9] Conversion of the drug into the 1-acid succinate,[10] 1-carbamate,[11] or 1-nicotinate[12] has been effected in an attempt to prolong its action by protecting the labile 1-hydroxy group, but these derivatives are not entirely satisfactory, so that a new long-acting ester might be of interest.

Chemistry.—The esters (listed in Table I) were prepared (Scheme I) by reaction of 1,2-epoxy-3-(*o*-toloxy)propane (**4**) with the appropriate acid (procedure A), or by reaction of mephenesin (**3**) with the appropriate acid chloride in pyridine solution (procedure B), or, in the case of the *o*-hydroxyphenoxyacetate (**7**), by reaction of the lactone **2** with **3** (procedure C). The *p*-amino esters were obtained from the corresponding nitro derivatives by catalytic hydrogenation.

Several attempts to prepare mephenesin mono-*o*-nitrobenzoate by procedure A were unsuccessful. Treatment of **3** with *o*-nitrobenzoyl chloride (procedure B) gave only the bisester **15**, even when **3** was used in excess. Treatment of **4** with *p*-nitrobenzoic acid gave

SCHEME I



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the monoester **11**, while attempts at preparing the same ester by procedure B gave, in analogy with the previous case, only the bisester **13**. Partial acid hydrolysis of **13** gave **11**, whereas **15** resisted attempts at hydrolysis. It was assumed that the primary ester was formed in all procedures. As a matter of fact, (a) ring opening of propylene oxide derivatives by acids occurs to give preferentially the primary ester, which may be formed directly, or through acyl migration;[13] (b) the primary rather than the less reactive secondary alcohol function of **3** should react with the acid chloride or with **2**. The proposed structure was confirmed through oxidation of the esters **6** and **11**, selected as representatives of the series: both compounds were readily transformed, under mild conditions, into the corresponding esters of 1-hydroxy-3-(*o*-toloxy)propan-2-one (**5**).

A study of the noncatalyzed hydrolysis of all esters was carried out in aqueous acetone at 100°, following the method described in ref 7. The half-life for the hydrolysis of **7** was *ca.* 5.5 hr (*vs. ca.* 6 hr for **1a**), while all other esters were practically unchanged after 24 hr under the same conditions. The facile hydrolysis of esters of **1b** was thus confirmed.

Pharmacology.—All compounds listed in Table I were screened for paralyzing activity in mice, at four dose levels, using groups of three male albino mice (Swiss SM) for each dose level. The animals were injected intraperitoneally at dose levels of 100, 200, 400, and 800 mg/kg, and observed for 2 hr. Only the phenoxyacetates **6–9** were found to possess mephenesin-like activity and were selected for further study. Mephenesin salicylate (**10**) was devoid of muscle relaxant activity at the 300–500-mg/kg levels, but exhibited a weak sedative action. The pharmacology of this compound is being investigated further in this laboratory. The esters **11–16** were practically devoid of mephenesin-like activity except at toxic doses (1000–1500 mg/kg). It is interesting to observe that oxidation of the active ester **6** to the corresponding ester of 1-hydroxy-3-(*o*-toloxy)propan-2-one (**17**) results in total loss of pharmacological activity. Compound **18** was also inactive. To our knowledge, 2-oxo derivatives of mephenesin had never been prepared and tested before.

In Table II are recorded the methods and results of a preliminary biological investigation on the four active compounds. Our intent was to provide comparative

(6) D. Gould, L. Finckenor, E. B. Hershberg, J. Cassidy, and P. L. Perlman, *J. Am. Chem. Soc.*, **79**, 4472 (1957).

(7) S. M. Kupchan and M. F. Saettone, *Tetrahedron*, **18**, 1403 (1962).

(8) P. Lebeau and G. Courtois, "Traite de Pharmacie Chimique," Vol. 2, Masson & Cie., Paris, France, 1946, p 1390.

(9) R. F. Riley and F. M. Berger, *Arch. Biochem.* **20**, 1959 (1949).

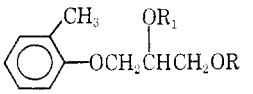
(10) F. M. Berger and R. F. Riley, *J. Pharmacol. Exptl. Therap.*, **96**, 269 (1949).

(11) (a) W. A. Lott and E. Pribyl, U. S. Patent 2,609,386 (1952); (b) F. M. Berger, *J. Pharmacol. Exptl. Therap.*, **104**, 468 (1952).

(12) T. I. Fand and R. H. Broh-Kahn, U. S. Patent 2,750,391 (1956).

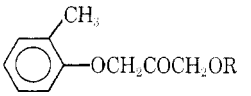
(13) A. Weissberger, "The Chemistry of Heterocyclic Compounds; Heterocyclic Compounds with Three- and Four-Membered Rings," Part 1, Interscience Publishers, Inc., New York, N. Y., 1964, pp 366–382.

TABLE I
MEPHENESIN ESTERS



Compd	R	R_1	Procedure[a]	Recrystn solvent[b]	Mp, °C[c]	Yield, %	Formula	Analyses[d]
6	$C_6H_5OCH_2CO$	H	A, B	Bz–PE	65–68	27 (A), 18 (B)	$C_{18}H_{20}O_5$	C, H
7	o-$HOC_6H_4OCH_2CO$	H	C	Bz–PE	109–110	28	$C_{18}H_{20}O_6$	C, H
8	p-$ClC_6H_4OCH_2CO$	H	A, B	Bz–PE	69–70	59 (A), 40 (B)	$C_{18}H_{19}ClO_5$	C, H
9	p-$FC_6H_4OCH_2CO$	H	A	Bz–PE	58–60, 67–70[e]	23	$C_{18}H_{19}FO_5$	C, H
10	o-HOC_6H_4CO	H	A	Bz–PE	81–84	33	$C_{17}H_{18}O_5$	C, H
11	p-$O_2NC_6H_4CO$	H	A	Bz	98–99	44	$C_{17}H_{17}NO_6$	C, H, N
12	p-$H_2NC_6H_4CO$	H	D	Bz	107–110	77	$C_{17}H_{19}NO_4$	C, H, N
13	p-$O_2NC_6H_4CO$	R	B	Bz–PE	145–147	32	$C_{24}H_{20}N_2O_9$	C, H, N
14	p-$H_2NC_6H_4CO$	R	D	Bz	130–135	51	$C_{24}H_{24}N_2O_5$	C, H, N
15	o-$O_2NC_6H_4CO$	R	B	Bz	97–100	30	$C_{24}H_{20}N_2O_9$	C, H, N
16	m,m-$(NO_2)_2C_6H_3CO$	H	A, B	Bz–PE	119–120	18 (A), 15 (B)	$C_{17}H_{16}N_2O_8$	C, H, N

OXIDATION PRODUCTS



17	$C_6H_5OCH_2CO$		E	99–101	50		$C_{18}H_{18}O_5$	C, H
18	p-$O_2NC_6H_4CO$		E	120–122	50		$C_{17}H_{15}NO_6$	C, H, N

[a] See Experimental Section. [b] Bz, benzene; PE, petroleum ether (bp 60–80°); E, ethanol. [c] Determined on a Kofler block and uncorrected. [d] Analytical results of the elements listed were within ±0.3% of the theoretical values. [e] The compound existed in two crystalline forms; both showed identical ir spectra in $CHCl_3$ solution and identical physiological activity.

TABLE II
PHARMACOLOGICAL EVALUATION OF THE PHENOXYACETATES PREPARED IN THIS STUDY[a]

Compd	ED_{50} (rotating rod)[b] mg/kg	ED_{50} (rotating rod)[b] mmol/kg	Duration of act.,[c] min (range)	Latency,[d] min	LD_{50}[e] mg/kg	LD_{50}[e] mmol/kg	Ratio LD_{50}/ED_{50}
Mephenesin	84	0.45	4 (3–6)	2	570	3.1	6.8
Mephenesin carbamate	125	0.55	7 (3–12)	4	600	2.7	4.9
6	284	0.94	14 (8–18)	7	1300	4.3	4.5
7	372	1.1	10 (5–15)	8	2800	8.4	7.6
8	325	0.92	23 (19–24)	8	950	2.7	2.9
9	275	0.82	17 (12–19)	9	950	2.8	3.4

[a] All compounds were administered by intraperitoneal injection as a suspension in physiological solution containing 3% Giten O (Polyoxyethylene Sorbitan Monooleate, A. & D. Treves Inc., New York, N. Y.). Male, albino Swiss S.M. mice, 25–30 g, to whom food had been withdrawn 24 hr prior to injection, were used in all tests. [b] The rotating rod test was performed as indicated by N. W. Dunham and T. S. Miya, *J. Am. Pharm. Assoc., Sci. Ed.*, **46**, 208 (1957), using 24–36 animals/compound. The reported values were obtained by graphical interpolation. [c] The duration of action is defined as the mean time, calculated by graphical interpolation, from onset of action to the final pass in the rotating rod test, for animals receiving a dose in the ED_{50} range. [d] The latency is defined as the mean time from time of injection to the onset of action (inability to remain on the rotating rod for 1 min). [e] The LD_{50} were calculated after 48 hr.

data, under equivalent conditions, on the intensity and duration of activity (rotating rod test; see footnote *b*, Table II) and on the toxicity of our compounds with respect to mephenesin and mephenesin carbamate.

Discussion

The results obtained with the esters **6–9**, and the relative pharmacological inertness of the other mephenesin esters prepared in this study, confirm the previously published observation[6] on aryloxyacetic acids and their inherent ability to impart a longer duration of pharmacologic activity to the respective drug esters. Indeed, the duration of action of mephenesin phenoxyacetates increases from 1.5 to 3 times over that of mephenesin carbamate. The intensity of action of the phenoxyacetate esters slightly decreases as compared with the carbamate ester, however. Interestingly, the testosterone aryloxyalkanoates have been reported[6] to have given maxima as high as three times that of the propionate and twice that of other conventional esters. In our compounds, the activity ranged from 0.5 to 0.75 that of the carbamate, on a molar basis. The fact that, of all four esters, the *o*-hydroxyphenoxyacetate **7** shows the shortest duration of action (10 *vs.* 23 min of the *p*-chlorophenoxyacetate) might be explained in terms of increased *in vivo* rate of hydrolysis, where intramolecular catalysis might play a small but definite role. However, the *in vivo* effect is not very significant, especially when compared with the *in vitro* results, and further studies are necessary to evidence it more accurately.

Of particular interest is the ester **7**, which shows the highest therapeutic index of the whole group. This is very probably due to the low toxicity of *o*-hydroxyphenoxyacetic acid (LD_{50} > 2500 mg/kg), which is the least toxic of the four acids. In conclusion, aryloxyacetic acids may be looked upon as interesting latentiating agents, particularly when compared with other acids. Further study dealing with the application of a wide series of these acids to the latentiation of drugs containing hydroxyl or amino groups is now in progress.

Experimental Section

Chemicals.—Phenoxyacetic acid and the corresponding acid chloride were prepared as described by Mameli, *et al.*[14] *o*-Hydroxyphenoxyacetic acid lactone (**2**) was prepared from the corresponding acid as described by Ludewig.[15] *p*-Chiorophenoxyacetic acid and the corresponding acid chloride were prepared according to Minton and Stephen.[16] 1,2-Epoxy-3-(*o*-toloxy)propane (**4**) was prepared as indicated by Chizhevskaya, *et al.*,[17] and was purified by distillation under reduced pressure, bp 132–135° (10 mm). All other starting materials were commercially available products, purified by crystallization to constant melting point.

Procedures for the Preparation of the Esters. A.—Compounds **6, 8–11,** and **16** were prepared from **4** and the appropriate acid, following the procedure given by Petrow, *et al.*,[18] for the preparation of mephenesin benzoate.

B.—Compounds **6, 8, 13, 15,** and **16** were prepared by treatment of mephenesin (**3**) with the appropriate acid chloride. Treatment of **3** with *o*- or *p*-nitrobenzoyl chloride gave only the bisester **15** and **13,** respectively, even when the reactants were used in equimolar amounts. As an example, the preparation of **8** is reported. A mixture of **3** (5.0 g, 27 mmoles), *p*-chlorophenoxyacetyl chloride (5.6 g, 27 mmoles), and anhydrous pyridine (10 ml) was heated 1 hr at 100°, then was poured into cold water. The mixture was extracted with ether, the ethereal extract was washed with water, 10% Na_2CO_3, and water, dried ($MgSO_4$), and evaporated to give an oil which crystallized from benzene–petroleum ether to afford pure **8** in 40% yield.

C.—The ester **7** was prepared by treatment of **3** with the lactone **2** as follows. A mixture of **3** (9.1 g, 0.05 mole) and **2** (7.5 g, 0.05 mole) was heated at 130° for 24 hr. The resulting syrupy material afforded, on crystallization from benzene–petroleum ether (bp 60–80°), 5.9 g (28%) of ester, which was purified by further crystallization from the same solvent mixture.

D.—Compounds **12** and **14** were obtained from **11** and **13,** respectively, by catalytic reduction over PtO_2 in dioxane, as follows. A solution of the compound (5.0 g) in anhydrous dioxane (80 ml) was hydrogenated at normal pressure until the theoretical amount of H_2 had been adsorbed. The catalyst was filtered off and the solvent was evaporated at reduced pressure; the oily residue was crystallized from anhydrous ether.

Oxidation of 6 and 11.—The oxidation of **6** and **11** to the corresponding 1-hydroxy-3-(*o*-toloxy)propan-2-one derivatives **17** and **18** was carried out as follows. To a solution of the compound (3.0 mmoles) in acetone (10 ml, previously distilled over $KMnO_4$) was added dropwise an 8 *N* solution of CrO_3 in H_2SO_4 (1.5 ml),[19] while stirring and cooling at 5°. The mixture was then diluted with H_2O, and the solid which separated was collected, washed with 10% Na_2CO_3 and H_2O, dried, and crystallized from EtOH.

Acid-Catalyzed Hydrolysis of 13 to 11.—A solution of **13** (1.0 g) in 95% EtOH (10 ml) was treated with several drops of concentrated HCl, then was heated 1 hr under reflux and poured into H_2O. The solid which separated gave on crystallization from C_6H_6 0.41 g (60%) of pure **11**,[20] mp 98–99°.

Kinetic Experiments. Hydrolysis of 6–11 in Aqueous Acetone.—The kinetic experiments were performed with an electrically controlled oil bath (100 ± 0.01°), using analytical grade acetone, purified by reflux over $KMnO_4$, desiccation over K_2CO_3, and fractionation.

Solutions (0.1 *M*) of the compounds in acetone containing 40% H_2O by volume were heated at 100° in sealed 10-ml ampoules. The rates of reaction were measured by titration of successive ampoules, removed after appropriate intervals, with standard alkali (phenol red indicator). The hydrolysis of **7** was found to be first order in ester up to 90% completion; $K = 3.5 \times 10^{-5}$ sec^{-1}, half-life *ca.* 5.5 hr. All other esters were not appreciably hydrolyzed after 24 hr.

Acknowledgment.—The authors wish to express their gratitude to Professor L. Beani, Institute of Pharmacology, University of Pisa, and to Dr. N. Tellini, Guidotti Pharmaceutical Laboratories, Pisa, for stimulating discussions.

(14) (a) E. Mameli, E. Gambetta, and G. Rimini, *Gazz. Chim. Ital.*, **50**, 170 (1920); (b) E. Mameli, *ibid.*, **56**, 763 (1926).

(15) H. Ludewig, *J. Prakt. Chem.*, **61**, 345 (1900).

(16) T. H. Minton and H. Stephen, *J. Chem. Soc.*, **121**, 1600 (1922).

(17) I. I. Chizhevskaya, Z. B. Idel'chik, L. A. Yakimovich, and K. S. Shadurski, *Vestsi Akad. Navuk Belaurusk. SSR, Ser. Fiz.-Tekh. Navuk*, 115 (1957); *Chem. Abstr.*, **52**, 9009 (1958).

(18) V. Petrow, O. Stephenson, and A. M. Wild, *J. Pharm. Pharmacol.*, **12**, 37 (1960).

(19) Prepared according to C. Djerassi, R. R. Engle, and A. Bowers, *J. Org. Chem.* **21**, 1547 (1956).

(20) The reaction very probably involves hydrolysis of the primary ester function, followed by acyl migration to give the α-monoester **11**. For similar cases of acyl migration in diol monoesters, see, *e.g.*, G. Berti, F. Bottari, and B. Macchia, *Ann. Chim.* (Rome), **52**, 1101 (1962).

Reduced Derivatives of Methotrexate[1]

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It has been reported that 5,6,7,8-tetrahydromethotrexate (III) is a more potent folic acid antagonist than methotrexate (I) for *Streptococcus faecalis*,[4] *Pediococcus cerevisiae*,[4] mice,[5] chicks,[6] and dogs.[7] When a method developed for separating dihydrofolate and tetrahydrofolate[8] was applied to III it was observed that the material was actually a mixture of dihydromethotrexate (II) and III. Some properties of the purified derivatives are reported here.

The reduced material showed two major peaks on diethylaminoethylcellulose chromatography. It was shown spectrally that the peak eluted first was III and the second II. They accounted for 39 and 52% of the total absorbing material, respectively. The absorption maxima are shifted 10 mμ toward longer wavelengths as compared with the corresponding aminopterin derivatives.[9] The extinction coefficients at maximum absorption were assumed to be the same as for aminopterin derivatives.[9]

III and II are less potent than I as inhibitors of dihydrofolate reductase but more potent as inhibitors of thymidylate synthetase (Table I). In every system tested II was more inhibitory than III. III is most likely a mixture of diastereoisomers resulting from the addition of hydrogen to carbon 6. The contribution of each diastereoisomer to the inhibition is not known.

Experimental Section

Compound I, provided by Lederle Laboratories, Pearl River, N. Y., was purified by diethylaminoethylcellulose chromatography as described for aminopterin.[9] Hydrogenation was carried out in AcOH using PtO_2 catalyst.[10] The reduced material was filtered under H_2 and washed with ether.[11]

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(4) R. L. Kisliuk, *Nature*, **188**, 584 (1960).

(5) J. A. R. Mead, J. M. Venditti, A. W. Schrecker, A. Goldin, and R. L. Kisliuk, *ibid.*, **189**, 937 (1961).

(6) R. L. Kisliuk and M. R. S. Fox, *Arch. Biochem. Biophys.*, **99**, 534 (1961).

(7) R. L. Kisliuk, J. Jankowski, and H. Kahaner, *Tufts Folia Med.*, **8**, 117 (1962).

(8) C. K. Mathews and F. M. Huennekens, *J. Biol. Chem.*, **235**, 3304 (1960).

(9) R. L. Kisliuk and M. D. Levine, *ibid.*, **239**, 1900 (1964).

(10) B. L. O'Dell, J. M. Vandenbelt, E. S. Bloom, and J. J. Pfiffner, *J. Am. Chem. Soc.*, **69**, 250 (1947).

(11) R. L. Kisliuk, *J. Biol. Chem.*, **227**, 805 (1957).