Synthesis and Estrogenic Properties of 17-*epi*-Ethynylestradiol and Its Ether Derivatives Epimestranol and Epiquinestrol

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The synthesis of 17-*epi*-ethynylestradiol (**10**), the 17β-ethynyl-17α-ol epimer of the well-known orally active estrogen, ethynylestradiol (**1**), was achieved by $LiAlH_4$ reduction of epoxide **9**, as well as by demethylating epimestranol (**11**) with CH_3MgI. Compound **11** was obtained by the unusual 17β-ethynylation of estrone 3-methyl ether **22** under equilibrating conditions. The in vitro estrogen receptor-binding affinity and the oral estrogenicity in the rat for the 17-*epi* compounds **10**, **11**, and **20** (epiquinestrol) was evaluated. Despite moderate estrogen receptor-binding affinity, compound **10** was devoid of measurable estrogenicity at 10 mg/kg or antiestrogenicity at 3 mg/kg.

17α-Ethynylestradiol (**1**), the first orally active synthetic estrogen,[1] has been known since 1938. The importance of **1** and its ether derivatives mestranol (**2**)[2] and quinestrol (**4**)[3] in oral contraception is well established.[4] The isomeric compound 17-*epi*-ethynylestradiol (**10**, 17β-ethynylestradiol), however, has not been described to date.[24] This paper describes the synthesis and estrogenic properties of **10** and its ether derivatives epimestranol (**11**) and epiquinestrol (**20**). Compound **11** was first obtained in our laboratories[5] as the unexpected epimerization product of mestranol acetate (**17**) on alumina. Its structure was confirmed by a three-step synthesis from **2** via **6** and **8**. Herein, we also report a direct synthesis of **11** from **22** via ethynylation, representing the first example of signficant 17β-ethynylation (~13%) of a 17-keto steroid not hindered by a neighboring α substituent.

Chemistry. Alkynylation of normal[6] 17-keto steroids generally proceeds by attack of the organometallic reagent from the less-hindered α side of the keto group, giving almost exclusively the 17β-alkynyl-17β-ol derivatives. Products resulting from 17β attack have been reported but in very low yields (0.25%) when the alkyne involved was acetylene (ethynylation)[7] and in significant yields only when either 17α attack was hindered by a neighboring α substituent[8,9] or when a substituted alkyne was invovled.[10,11]

The hindered ketone, camphor, is normally ethynylated in a highly stereospecific manner (95% exo-OH) like 17-keto steroids. However, an appreciable quantity of the abnormal isomer is formed (42% endo-OH) under equilibrating ethynylation conditions.[12] This prompted us to try the ethynylation of **22** under similar conditions with the aim of obtaining **11** in one step.[13] Accordingly, **22** was treated with a continuous stream of acetylene in *N*-methylpyrrolidone in the presence of a large excess of powdered KOH (8 equiv) for 63 h at room temperature. TLC analysis revealed the presence of unreacted **22** and the epimeric ethynylcarbinols **2** and **11**. Ketone **22** was removed as its corresponding semicarbazone derivative, and the residuals **2** and **11** (70%, 87:13 by quantitative TLC) were separated by column chromatography on silica gel [**11** (7%) and **2** (49%)]. A similar attempt to ethynylate free phenol **21**, however, afforded only the normal product **1** without any detectable amount (TLC) of epimer **10**. The more facile 17β-ethynylation observed with the 3-methoxy compound **22** compared to its 3-phenolic congener **21** is reminiscent of similar behavior noted with a 3-methoxy-16α-acetoxy-17-keto steroid and its 3-phenolic (or acetoxy) analogue.[14] In the latter case, however, the principal factor governing 17β approach is the steric hinderance to 17α approach due to the 16α-acetoxy group.

The synthesis of **10** was accomplished by two routes (Scheme I). When the epoxide **9**, obtained by *m*-CPBA treatment of the Δ-16-enyne **7**,[15] was reduced with $LiAlH_4$, epimer **10** was obtained in low yield (11%). The major products of this reduction were the Δ-16-enyne **5** and a mixture of more polar products presumed to be the isomeric 17-ethynyl-16α-ols **12** and **13**. This has analogy in the formation of alcohols[24,25] **14** and **15** during the reduction of epoxide **8** to **11**.[5] Compound **10** could be prepared more efficiently (~70%) by demethylating **11** by fusion with CH_3MgI[16] at 175 °C. To find a shorter method for preparing **10**, the monoacetate **16** and the diacetate **18** were treated with alumina under the conditions that epimerized **17** to **11**.[5] However, no **10** was observed, but a mixture of the starting materials and their hydrolysis products (free phenols) were recovered. This was not totally surprising in view of our earlier findings[17,18] that the presence of strongly polar functional groups, even when remotely located in ring A, inhibited or prevented the alumina-induced deacetoxylation of several steroidal tertiary 17β-acetates.

The 3-cyclopentyl ether **20** was prepared in the usual manner by reacting **10** with cyclopentyl bromide and K_2CO_3 in EtOH.

Biological Activity. Table I summarizes the oral estrogenic properties as determined by the standard uterotropic assay in immature rats[19] and vaginal cornification values in ovariectomized, mature rats.[20] The compounds were also evaluated for their in vitro binding affinity (Table I) for the rabbit uterine estrogen receptor (see Experimental Section).[21]

By inspection of the dosages of compounds which caused between 0 and 100% vaginal cornification in treated rats, the compounds could be ranked in the same order of potency as indicated by the uterotropic assay. All the 17-*epi* compounds (**10**, **11**, and **20**) were very weak estrogens in both assays and had moderate (**10**) or no measurable affinity (**11** and **20**) for the estrogen receptor compared to their 17α-ethynyl-17β-ol counterparts (i.e., **1**, **2**, and **4**, respectively). The augmented estrogenicity of 3-cyclopentyl ether derivative **20** compared to 3-phenol **10** in the 17-*epi* series has its parallelism in the regular series (**4** and **1**). However, surprising was the extremely low estrogenicity of **10**, such that it failed to elicit any uterotropic response even at 10 mg/kg, even though some weak response would be expected on the basis of its moderate binding affinity for the estrogen receptor. This observation suggested that **10** might have useful antiestrogen properties. However, the compound was inactive as an antiestrogen[22] when tested up to 3 mg/kg. One possible explanation of these results is that **10** could be rapidly me-

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Scheme I

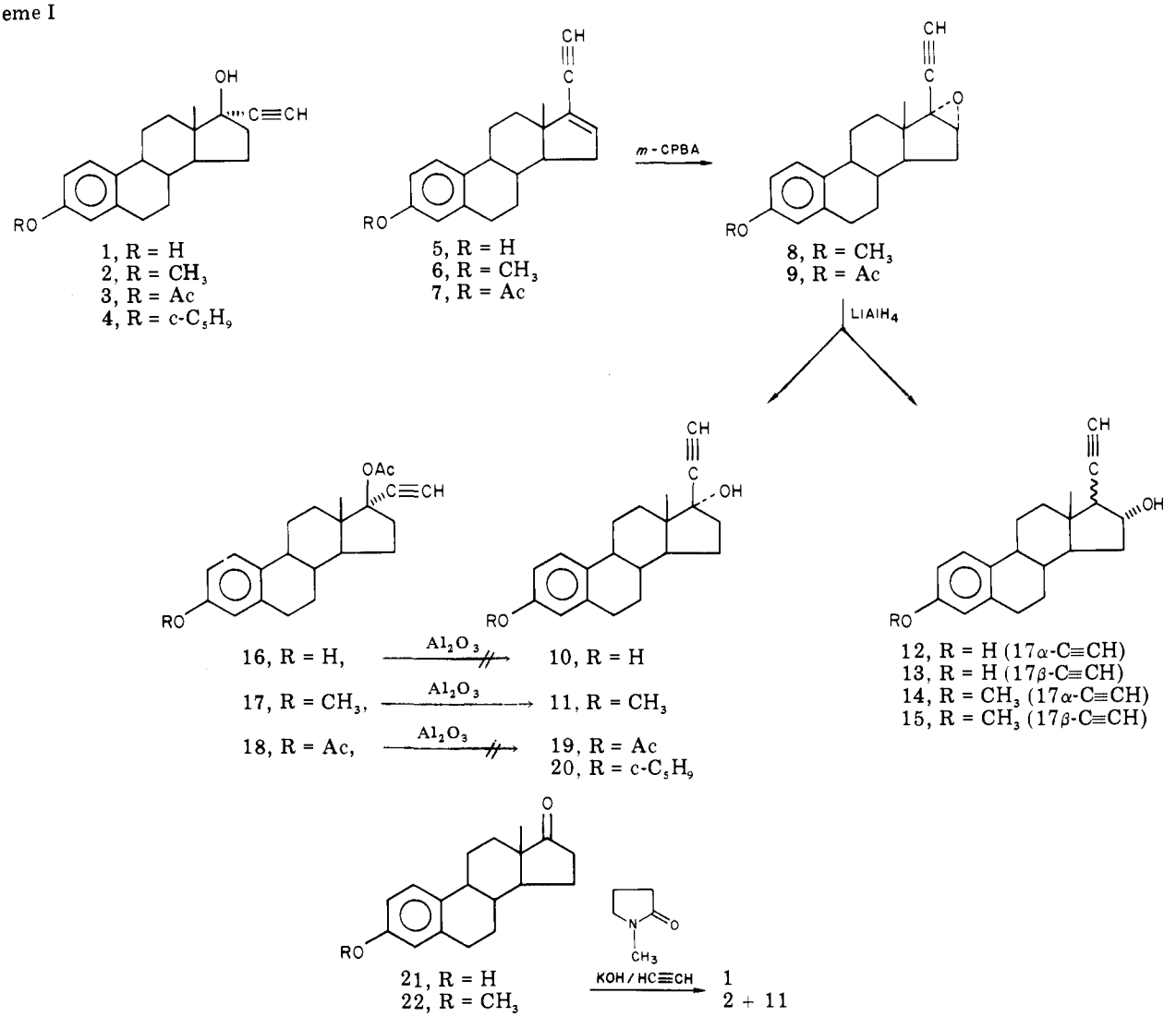

Table I. Results of Biological Assays

no.	estrogen receptor[a] rel binding affinity	estrogen receptor[a] IC_{50}, M	uterotropic response rel potency	uterotropic response 95% CL	vaginal cornification dose range, mg/kg	vaginal cornification % of rats exhib vaginal cornification
1	1.0	2.4×10^{-10}	1.00		0.025-0.100	10-100
2	0.0012	2.0×10^{-7}	0.84[c]	0.76-0.94	0.025-0.100	11-80
4	0.00004	6.0×10^{-6}	1.66	1.36-2.04	0.005-0.010	40-100
10	0.016	1.5×10^{-8}	*e*		5.0-10.0	0-22
11		*b*	0.0023[d]	0.0005-0.0074	10.0-20.0	0-75
20		*b*	0.050	0.038-0.073	0.25-0.5	25-90

[a] The results are the means from two assays. The IC_{50} is the concentration which caused 50% depression in the binding of the 17β-[^{3}H]estradiol to the receptor. [b] IC_{50} was not reached by 1×10^{-4} M. [c] The results are the mean potency and 95% confidence limits resulting from the statistical combination of four assays. [d] The results are the mean potency and 95% confidence limits from the statistical combination of three assays. [e] Minimal stimulation at 10 mg/kg.

tabolized so that insufficient quantities of the compound reach the estrogen receptor sites to cause estrogenic or antiestrogenic activities in vivo.

Experimental Section

Chemical Syntheses. Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. UV spectra were determined on a Cary Model 11 recording spectrophotometer in ethanol, IR spectra were recorded on a Unicam SP1000 infrared spectrophotometer in KBr pellets, and NMR spectra were obtained on a Varian A-60 spectrometer using $CDCl_3$ as the solvent with tetramethylsilane as the internal standard unless otherwise indicated. Optical rotations were determined on a Rudolph Model 70 polarimeter attached to a Model 200 photoelectric unit. TLC analyses were performed on Uniplate SilicAR 7GF (Analtech Inc.). All evaporations were carried out in vacuo. Symbols of elements refer to microanalyses with results within ±0.4% of calculated values.

3-Methoxy-19-norpregna-1,3,5(10)-trien-20-yn-17α-ol (Epimestranol, 11). A. Ethynylation of 22. Acetylene was continuously bubbled at room temperature for 63 h through a solution of **22** (2.84 g, 10 mmol) and powdered KOH (5 g, 88 mmol) in *N*-methylpyrrolidinone (60 mL). The mixture was poured into ice-water and extracted with CH_2Cl_2. The organic layer was washed with 10% aqueous HCl and H_2O, dried (Na_2SO_4), and evaporated to dryness. The residue was treated in CH_3OH with semicarbazide hydrochloride (2.5 g) and pyridine and refluxed for 1 h. The semicarbazone (0.6 g, 16%), obtained upon ice

cooling, was removed by filtration and the filtrate evaporated to dryness. The residue was dissolved in CH_2Cl_2, washed with 10% aqueous HCl and H_2O, and dried (Na_2SO_4), and the solvent was removed to afford a mixture of **2** and **11** (2.15 g, 69%). Quantitative TLC analysis (SilicAR 7GF, 5% EtOAc/benzene) indicated the presence of **11** (13%, R_f 0.46) and **2** (87%, R_f 0.38). Column chromatography on silica gel with 2–5% EtOAc/benzene afforded **11** (0.21 g, 7%, mp 135–136 °C, reported[5] 136–137 °C) and **2** (1.53 g, 49%, mp 150–151 °C, reported[2] 150–151 °C).

B. Reduction of 8 with $LiAlH_4$. Reduction of **8** (10 g, 32.5 mmol) with $LiAlH_4$ (10 g) in refluxing THF (800 mL), followed by chromatography on silica gel (1% EtOAc/benzene), afforded **11** (1.5 g, 15%, mp 136–137 °C)[5] in addition to the less polar enyne **6** (4.42 g, 47%). Further elution (1–2% EtOAc/benzene) afforded two polar products[24] characterized as follows: Compound **14**: yield 2.5 g (25%); mp 136–140 °C; $[\alpha]^{25}_D$ −6.96° (*c* 1, $CHCl_3$); IR 2.8, 3.01 μm; NMR δ 0.82 (s, 3, 18-CH_3), 2.43 (d, J = 2.0 Hz, 1, C≡CH), 2.94 (dd, J_1 = 1.0, J_2 = 2.5 Hz, 17β-H), 3.75 (s, 3, OCH_3), 4.5 (br m, 1, 16β-H); UV (EtOH) λ_{max} 278 nm (ε 2060), 287 (1930). Anal. ($C_{21}H_{26}O_2$) C, H. Compound **15**: yield 0.57 g (0.57%); mp 208–210 °C; $[\alpha]^{25}_D$ +68° (*c* 1, $CHCl_3$); IR 2.8, 3.01 μm; NMR δ 0.86 (s, 3, 18-CH_3), 2.22 (d, J = 1.5 Hz, C≡CH), 2.23 (dd, J = 2.0 Hz, 17α-H), 3.78 (s, 3, OCH_3), 4.44 (br m, 16β-H). Anal. ($C_{21}H_{26}O_2$) C, H.

16α,17α-Epoxy-19-norpregna-1,3,5(10)-trien-20-yn-3-ol Acetate (9). A solution of *m*-CPBA (5.12 g, 50 mmol) in $CHCl_3$ (200 mL) was slowly added over a period of 0.5 h to a stirred solution of **7** (8.0 g, 25 mmol) in $CHCl_3$ (100 mL). After stirring the solution for an additional 2.5 h, the excess peracid was destroyed by the addition of a saturated solution of $NaHSO_3$. The $CHCl_3$ layer was washed successively with aqueous 10% $NaHCO_3$ and H_2O, dried (Na_2SO_4), and evaporated. The residue was chromatographed on silica gel (300 g) with benzene/hexane to afford **9** (5.66 g, 66%). Recrystallization from CH_3OH gave 3.4 g of **9** (40%, mp 153–155 °C), which upon crystallization from ether/hexane gave an analytical sample: mp 154–155.5 °C; $[\alpha]^{24}_D$ +9.1 (*c* 0.5, $CHCl_3$); UV λ_{max} 275 nm (ε 747); IR 3.03, 3.31 (16α-H), 5.72 μm; NMR δ 0.92 (s, 3, 18-CH_3), 2.25 (s, 3, CH_3COO), 2.40 (s, 1, C≡CH), 3.63 (s, 1, 16β-H). Anal. ($C_{22}H_{24}O_3$) C, H.

19-Norpregna-1,3,5(10)-trien-20-yne-3,17α-diol (10). A. Demethylation of 11 with CH_3MgI. A 2.6 M ethereal solution of CH_3MgI (278 mL, 723 mmol) was charged to a 2-L, three-necked round-bottomed flask equipped with a magnetic stirrer, an argon inlet tube, and an air condenser, followed by **11** (16 g, 51 mmol). The mixture was slowly heated in an oil bath to 175 °C and maintained at that temperature for 3 h. The reaction flask was then cooled in a dry ice–acetone bath, a mechanical stirrer was introduced to break up the solidified foamy mass, and a saturated solution of NH_4Cl (500 mL) was carefully added (**highly exothermic!**). The mixture was adjusted to pH 5 with concentrated HCl and extracted with EtOAc. The organic layer was washed (H_2O) and dried (Na_2SO_4), and the solvent was evaporated to afford a crystalline residue (16 g). Chromatography on a column of SilicAR (3–5% EtOAc/benzene) afforded **10** (10 g, 65%, mp 207–210 °C). Recrystallization from acetone/hexane (charcoal treatment) yielded analytically pure **10**: yield 7.9 g (52%); mp 210–212 °C; $[\alpha]^{20}_D$ +68° (*c* 1.0, dioxane); IR 2.9, 3.03 μm; NMR ($CDCl_3/CD_3OD$) δ 0.89 (s, 3, 18-CH_3), 2.53 (s, 1, C≡CH), 6.5–7.6 (m, 3, Ar H). Anal. ($C_{20}H_{24}O_2$) C, H.

B. Reduction of 9 with $LiAlH_4$. A solution of epoxide **9** (100 mg) in ether (20 mL) was added dropwise to a suspension of $LiAlH_4$ (100 mg) in dry ether (50 mL), and the mixture refluxed for 24 h. After cooling the mixture, excess $LiAlH_4$ was decomposed by adding EtOAc (2 mL), followed by the addition of a saturated solution of Na_2SO_4 and solid CO_2. The mixture was then extracted with EtOAc, the organic layer was dried (Na_2SO_4), and the solvent was evaporated to afford a residue (80 mg). Purification by preparative TLC (Analtech PQIF, 10% EtOAc/benzene) afforded three principal products. The least polar material (25 mg, 30%) was identified as the enyne **5**: mp 166–170 °C; NMR δ 0.88 (s, 3, 18-CH_3), 3.10 (s, 1, C≡CH), 6.15 (m, 1, 16-H). The more polar band (10 mg, 11%) was presumably a mixture of **12** and **13**. The middle band was epimer **10** (10 mg, 11%), mp 207–210 °C, identical by spectral analysis and TLC with the product obtained by method A.

3-Cyclopentoxy-19-norpregna-1,3,5(10)-trien-20-yn-17α-ol (Epiquinestrol, 20). A mixture of **10** (6.0 g, 20.27 mmol), anhydrous K_2CO_3 (8 g), and cyclopentyl bromide (12 g) in ethanol (200 mL) was heated at reflux under N_2 for 4 h. The solvent was evaporated and the residue partitioned between H_2O and ether. The organic layer was washed (brine) and dried (Na_2SO_4), and the solvent was evaporated. The residue was purified by chromatography on silica gel (400 g). Elution with 0.5% EtOAc/benzene afforded **20**: mp 113–114 °C; yield 6.0 g (82%). One recrystallization from MeOH afforded analytically pure **20**: yield 5.0 g (68%); mp 114–115 °C; $[\alpha]^{25}_D$ +62.5° (*c* 1, $CHCl_3$); IR 2.79, 3.01 μm; NMR δ 0.88 (s, 3, 18-CH_3), 2.49 (s, 1, C≡CH), 4.75 (br m, 1, $OCHC_4H_8$). Anal. ($C_{25}H_{32}O_2$) C, H.

Biological Procedures. Uterotropic Activity. Following the standard procedure[19] in immature rats, the test was conducted as six point assays (three doses of standard **1** and three doses of each test compound administered orally in sesame oil) using 9–15 rats per dose group. Dose–response curves were analyzed for linearity and parallelism, and the relative potency estimate and 95% confidence limits were computed.[23]

Vaginal cornification studies were conducted in ovariectomized, mature rats by the Allen–Doisy procedure[20] using 14–59 rats per compound (4–20 per group). Dose ranges were chosen on the basis of results in the uterotropic studies. Vaginal smears were considered to be positive if they consisted of predominantly nucleated or cornified epithelial cells and no leucocytes. Results are expressed as the dose ranges which produced positive smears.

Antiestrogenic activity was determined by a modification of the method of Edgren and Calhoun[22] in immature rats. The test material was administered orally for 3 days together with sufficient **1** to cause a doubling of the uterine weight.

In vitro estrogen receptor binding affinities were determined by a modification of the procedure of Hahn et al.,[21] using rabbit uterine cytosol preparation. Each reaction mixture contained 0.7 μg of protein from the receptor preparation and 16 000 dpm of 17β-[^{3}H]estradiol (specific activity 113 Ci/mmol) in 0.25 mL of 0.01 Tris-HCl buffer, pH 8.0, containing 0.001 M ethylenediaminetetraacetic acid and 0.25 M sucrose. Each compound was tested in a dose–response fashion using eight concentrations from 1×10^{-10} to 1×10^{-4} M in duplicate. After incubation at 4 °C for 16–18 h, the compounds bound to the receptor were separated from those free in solution using dextran-coated charcoal. The amount of radioactivity bound to the receptor was quantified in Aquasol using a liquid scintillation spectrometer, and the results were compared to standard compounds.

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References and Notes

(1) H. H. Inhoffen, W. Logemann, W. Hohlweg, and A. Serini, *Chem. Ber.*, **71**, 1024 (1938).
(2) F. B. Colton, U.S. Patent 2 666 769 (1954).
(3) A. Ercoli and R. Gardi, *Chem. Ind. (London)*, 1037 (1961).
(4) Compounds **1** and **2** are the only two estrogens currently used in the available oral contraceptive preparations. Compound **4**, a potent long-acting oral estrogen, was investigated as a component of a potential "one-a-month" contraceptive pill: R. B. Greenblatt, *Fertil. Steril.*, **18**, 207 (1967).
(5) R. M. Kanojia, L. Yarmchuck, and I. Scheer, *J. Org. Chem.*, **39**, 2304 (1974).
(6) L. F. Fieser and M. Fieser, "Steroids", Reinhold, New York, 1959, p 467.
(7) T. Reichstein and C. Meystre, *Helv. Chim. Acta*, **22**, 728 (1939).
(8) J. Hannah and J. H. Fried, *J. Org. Chem.*, **29**, 3739 (1964).
(9) N. W. Atwater, R. H. Bible, Jr., E. A. Brown, R. R. Burtner, J. S. Mihina, L. N. Nysted, and P. B. Sollman, *J. Org. Chem.*, **26**, 3077 (1961).
(10) T. C. Miller and R. G. Christiansen, *J. Org. Chem.*, **32**, 2781 (1967).
(11) G. Muller, J. Mathieu, A. Petit, and L. Velluz, *Bull. Soc. Chim. Fr.*, 747 (1951).
(12) M. L. Capmau, W. Chodkiewicz, and P. Cadiot, *Tetrahedron Lett.*, 1619 (1965).

(13) The formation of trace amounts of 11 during ethynylation of 22 would not be surprising. However, neither the method of isolation nor any other physical properties of 11 were described when O. Engelfried et al. reported[14] the NMR chemical shifts of the C_{18} and ethynyl protons for 11.
(14) O. Engelfried, H. Gibian, F. Newman, K. Prezewowsky, G. Schutz, and R. Wiechert, *Arzneim.-Forsch.*, **16**, 1518 (1966).
(15) E. Ghera, *J. Org. Chem.*, **33**, 1042 (1968).
(16) A. L. Wilds and W. B. McCormack, *J. Am. Chem. Soc.*, **70**, 4127 (1948).
(17) R. M. Kanojia, S. Rovinsky, and I. Scheer, *J. Chem. Soc. D*, 1581 (1971).
(18) R. M. Kanojia, S. Rovinsky, L. Yarmchuck, and I. Scheer, Abstracts, IUPAC Meeting, 23rd, Boston, Mass., July, 1971.
(19) H. D. Lauson, C. G. Heller, J. B. Golden, and E. L. Sevringhaus, *Endocrinology*, **24**, 35 (1939).
(20) E. Allen and E. A. Doisy, *J. Am. Med. Assoc.*, **81**, 819 (1923).
(21) D. W. Hahn, J. L. McGuire, F. C. Greenslade, and G. D. Turner, *Proc. Soc. Exp. Biol.*, **137**, 1180 (1971).
(22) R. A. Edgren and D. W. Calhoun, *Proc. Soc. Exp. Biol. Med.*, **94**, 537 (1957).
(23) D. Finney, "Statistical Methods in Biological Assays", 3rd ed, Cambridge University Press, New York, 1971.
(24) R. M. Kanojia, U.S. Patents 3983112 (1976) and 4022892 (1977). A recent report by van Dijck et al.[25] describes compounds 14 and 15. The properties reported were in excellent agreement with our data, except for their specific rotation of 14 (-84.2°). Subsequently, in a private communication the authors[25] have confirmed the correctness of our value.
(25) L. A. Van Dijck, B. J. Lankwerden, and J. G. C. M. Vermeer, *Recl. Trav. Chim. Pays-Bas*, **96**, 200 (1977).

5-(α-Bromoacetyl)-2′-deoxyuridine 5′-Phosphate: An Affinity Label for Thymidylate Synthetase

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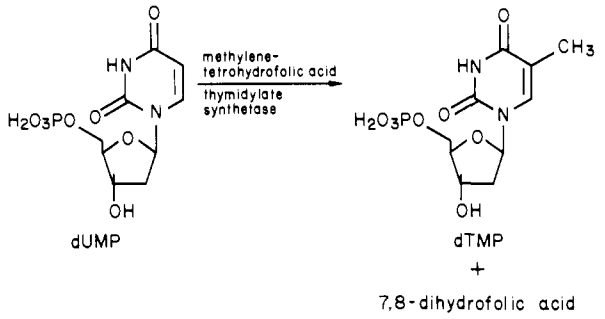
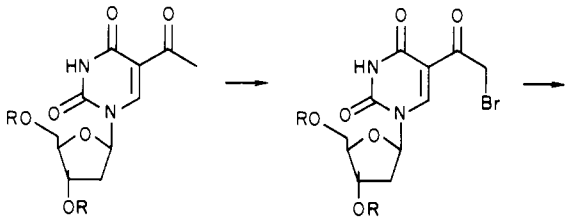
5-(α-Bromoacetyl)-2′-deoxyuridine 5′-phosphate (1) is an active-site-directed irreversible inhibitor of thymidylate synthetase from *Lactobacillus casei*. Analysis of the rate of inactivation of the enzyme in the presence of substrate confirmed the intermediate formation of a reversible enzyme–inhibitor complex.

Thymidylate synthetase (EC 2.1.1.45) catalyzes a two-step reductive alkylation of 2′-deoxyuridine 5′-phosphate (dUMP) to give thymidine 5′-phosphate (dTMP).[1] Clinical



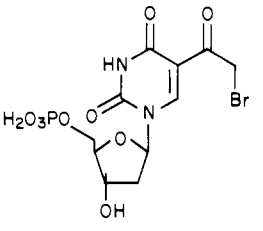
control of cancer growth and viral infection by inhibition of this enzyme has been realized using analogues of either the substrate or product substituted in the 5 position with strong electron-withdrawing groups such as fluoro or trifluoromethyl.[2] In addition to the inherently high enzyme affinity noted for 5-fluoro- and 5-(trifluoromethyl)-dUMP, they irreversibly inactivate thymidylate synthetase.

Recent studies of the mechanism of the first step in thymidylate synthetase catalysis have suggested that the sequence of the reaction is the addition of a cysteine SH group at the active site of the enzyme to carbon-6 of the pyrimidine ring in the substrate dUMP.[3,4] Subsequent steps in the reaction are the addition of the ES covalent complex to the cofactor, followed by rearrangement of the ternary complex to give the product dTMP and the oxidized cofactor 7,8-dihydrofolic acid.[1] A detailed analysis of the reaction requires the interaction of a second nucleophilic group at the active site that functions in abstraction of the carbon-5 proton of the substrate. With this background, the title compound (1) was designed as a mechanism based affinity label for thymidylate synthetase. Three features were embodied in this design: (1) substrate analogy, which should promote enzyme specificity; (2) the carbonyl (acetyl) at carbon-5 of the substrate, because of its inherent electron affinity, should enhance the Michael addition reaction whereby the enzymatic cysteine SH group adds to carbon-6 of the substrate; and (3) a bromo substituted α to a carbonyl group is recognized as a chemically reactive function that interacts with nucleophiles to give covalent bond formation. Thus, 5-(α-bromoacetyl)-2′-deoxyuridine 5′-phosphate (1) could be a



2, R = COC_6H_4-*p*-CH_3
4, R = H

3, R = COC_6H_4-*p*-CH_3
5, R = H



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substrate competitive inhibitor of thymidylate synthetase by virtue of its analogy to the substrate, the active-site cysteine SH group could reversibly add to carbon-6, and, finally, a second active-site nucleophile could displace the α-bromo group to give a covalent enzyme–inhibitor complex and the resultant enzyme inactivation. Preliminary results have been reported that suggest 1 is an affinity labeling reagent for thymidylate synthetase.[5]

The initial approach to the synthesis of 5-(α-bromoacetyl)-2′-deoxyuridine 5′-phosphate (1) was by bromination of the protected 5-acetyl nucleoside[6] 2 in acetic acid. Under similar conditions, 5-bromouracil is formed in 95% yield from 5-acetyluracil.[7] However, the protected nu-

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