3 is quite measureable. Yet in the gas phase, having nearly the same ϵ as cyclohexene, no reaction leading to lactone takes place at temperatures up to 450° (and under conditions which leave the lactone unchanged). These data, taken in conjunction with earlier stereochemical and tracer studies,[3] have therefore established that reaction occurs in solution *via* participation by the neighboring carbalkoxyl group with development of a mesomeric ion pair resembling **1**.

Clearly the ion pair formed in the gas phase does not possess the same structure (as its solution analog) in that the counterion, Br^-, is stringently localized to the region of the carbon atom with which it was covalently associated in the substrate. Therefore the only reaction possible is that in which Br^- abstracts the β-hydrogen in an elimination product-forming step (rather than the lactone-forming step in which it customarily engages when free in solution). However, in light of our observation that purely thermal elimination of HBr from **1** does *not* occur with a notable rate increase as compared to *n*-butyl bromide, it can also be said that ionization of the C–Br bond in **1** in the gas phase does not realize any benefit from neighboring group participation.

It may be noted, as well, that an ionization process, taking place in solution through a cyclic transition state such as **2**, should develop more readily in the gas phase, *if solvation were not a requirement for such ionization.* A polar, cyclic transition state is not resisted by a large negative entropy of activation if it is formed in the absence of solvent. That is to say, $\Delta F_{el} = \Delta H_{el}$ and $\Delta S_{el} = 0$ in the gas phase, where the subscript el designates the electrical parameters.[5] We must therefore conclude that the quasiheterolytic transition state for gas-phase dehydrohalogenation described by Maccoll[1] does not result in an ion-pair intermediate resembling the array of somewhat loosely bound counterions characteristic of the solution-phase heterolysis. Rather it should now be assumed that a considerably smaller degree of ionization may occur upon extension of the carbon–halogen bond in the absence of solvation which cannot be adequately calibrated by reference to the corresponding event in the solution-phase reaction.

Acknowledgment. We are greatly obliged to Professor P. D. Bartlett for very valuable suggestions in connection with the preparation of this manuscript.

(5) For a discussion of the evidence supporting this conclusion see A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," 2nd ed, John Wiley and Sons, Inc., New York, N. Y., 1961, and E. A. Moelwyn-Hughes, *Proc. Roy. Soc.* (London), **A155**, 308 (1936).

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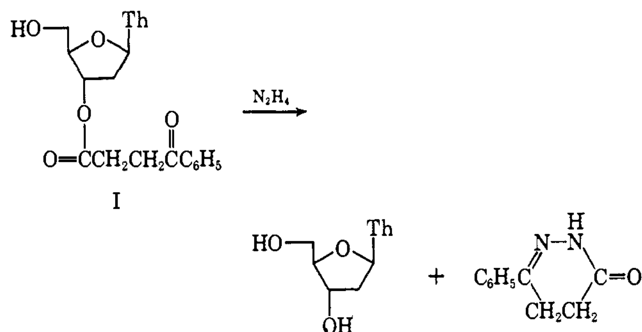
Oligonucleotide Syntheses Utilizing β-Benzoylpropionyl, a Blocking Group with a Trigger for Selective Cleavage[1,2]

Sir:

For synthetic work with nucleosides a blocking agent for hydroxyl groups was needed which was stable in pyridine solutions of arenesulfonyl chlorides and in aqueous pyridine yet could be removed efficiently at will under essentially neutral conditions. We have found that the benzoylpropionyl group satisfies these stipulations and is a very promising agent for protecting hydroxyl groups in polyfunctional compounds in general that are sensitive to acidic and basic reagents. Unblocking is accomplished by treatment at room temperature with hydrazine hydrate in pyridine buffered with acetic acid.[3] The keto function serves as a trigger for the cleavage, reacting selectively with the added reagent, hydrazine, to form an intermediate in which the nucleophilic NH_2 is favorably positioned to attack the neighboring ester. Under the reaction conditions hydrazine does not attack the heterocyclic rings of thymidine, deoxycytidine, deoxyadenosine, and deoxyguanosine,

The properties of the group are illustrated by the chemistry of compounds I and II. 3′-O-Benzoylpropionylthymidine (I), mp 158–160°, was obtained in 78% yield by esterification of 5′-O-di-(*p*-methoxytrityl)-thymidine with β-benzoylpropionic acid and dicyclohexylcarbodiimide in pyridine[4] and subsequent hydrolysis of the dimethoxytrityl ether with 80% aqueous acetic acid. On treatment with 0.5 *M* hydrazine in pyridine–acetic acid (80:20 v/v) for 3 hr this ester was converted quantitatively to thymidine and 4,5-dihydro-6-phenylpyridazone. Under these conditions O-acetyl groups



are not affected. Compound II was obtained in 63% yield from 1.0 mmole of 5′-O-monomethoxytritylthymidine and 1.5 mmoles of I by the method used for preparation of β-cyanoethyl derivatives of dinucleoside phosphates[5] (I used in place of thymidine). Both I and II gave satisfactory C, H, and N analyses. Each of the blocking groups in II could be removed independently, leaving the other two intact. Thus 80% aqueous acetic acid (10 min at steam-bath temperature) selectively cleaved the methoxytrityl ether, ammonium hydroxide (1 min at room temperature) selectively eliminated the cyanoethyl group, and hydrazine in pyridine–acetic acid (3 hr at room temperature) selectively removed the benzoylpropionyl group. The product of the hydrazine reaction serves as a useful intermediate in the stepwise

(1) Part X in a series on Nucleotide Chemistry. Part IX: T. Shimidzu and R. L. Letsinger, *J. Org. Chem.*, in press.

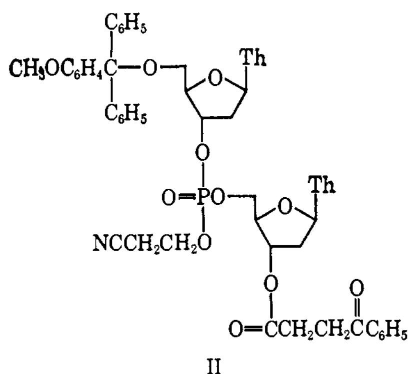
(2) This research was supported by the Division of General Medical Sciences, National Institutes of Health, GM-10265, and by Public Health Service Predoctoral Fellowships from the Division of General Medical Science awarded to M. H. C. (1-F1-GM23,558) and P. S. M. (5F1-GM34,033).

(3) T. Curtius reported in 1895 (*J. Prakt. Chem.*, [2] **50**, 529 (1895)) that equimolar amounts of ethyl β-benzoylpropionate and hydrazine hydrate react exothermically when mixed to yield 4,5-dihydro-6-phenylpyridazone. The potential of the β-benzoylpropionyl group as a blocking agent for alcohols, however, has apparently hitherto been overlooked.

(4) Attempts to prepare the acid chloride of benzoylpropionic acid for use in the esterification were unsuccessful as a consequence of the facile conversion of the acid to the unsaturated lactone.

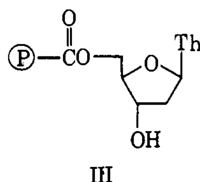
(5) R. L. Letsinger and K. K. Ogilvie, *J. Am. Chem. Soc.*, **89**, 4801 (1967).

synthesis of oligonucleotides with 3′–5′ phosphodiester links.



II

With the aid of the benzoylpropionyl blocking group an improved procedure was developed for synthesis of oligothymidylate derivatives on insoluble polymer supports.[6] Thymidine joined through the 5′-oxygen to an insoluble popcorn polystyrene polymer (III) was treated successively with an excess of each of the following reagents in pyridine: (1) pyridinium β-cyanoethyl phosphate and mesitylenesulfonyl chloride, (2) 3′-O-benzoylpropionylthymidine and 2,4,6-triisopropylbenzenesulfonyl chloride,[7] and (3) 0.4 *M* hydrazine (in the pyridine–acetic acid mixture). At each stage the polymer was separated from solvent and reagents by centrifugation and washed thoroughly. Repetition of steps 1–3 followed by cleavage of the oligonucleotide products from the support with sodium hydroxide in dioxane–water yielded thymidylyl-(3′–5′)-thymidylyl-(3′–5′)-thymidine (TpTpT), which was purified by chromatography on



III

DEAE-cellulose. The over-all yield based on thymidine bound to the support was 78%, an average of 95% for each of the chemical steps involved. Since some mechanical losses in handling the polymer undoubtedly occurred, the actual yield must have been higher. The product was completely degraded by snake venom phosphodiesterase.

(6) R. L. Letsinger and V. Mahadevan, *J. Am. Chem. Soc.*, **87**, 3526 (1965); **88**, 5319 (1966); R. L. Letsinger, M. H. Caruthers, and D. M. Jerina, *Biochemistry*, **6**, 1379 (1967).
(7) R. Lohrman and H. G. Khorana, *J. Am. Chem. Soc.*, **88**, 829 (1966).

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Studies on the Directive O-Methylation of Catechol Estrogens

Sir:

2-Hydroxyestrone, the principal metabolite of the estrogenic hormone estradiol, is converted *in vivo* to the 2-methyl ether without any evidence for formation of the 3-methoxy compound.[1,2] This is particularly significant because the two phenolic groups have virtually indistinguishable chemistry. Methylation *in vivo* of the important catecholamines leads nearly exclusively to the *m*-methyl ether.[3] In sharp contrast with these results in the living animal, *in vitro* methylation of catechols by rat liver O-methyl transferase enzyme is relatively indiscriminate and yields mixtures of monomethyl derivatives, often in approximately equivalent amounts.[1,4,5] The present study was undertaken to explore the possibility that sulfate conjugation, as a prior reaction stage, might be involved in the high selectivity of *in vivo* methylation.

The lack of selectivity for methylation of estrogen catechols *in vitro* was first confirmed by incubation of 200 μg of 2-hydroxyestradiol with a rat liver homogenate. Fresh rat liver was homogenized in ice-cold 0.25 *M* sucrose solution to a final concentration of 20%. The homogenate was centrifuged for 15 min at 1000*g* and the supernatant was used for the studies. The incubation medium contained 5 ml of homogenate, 1 ml of 0.1 *M* phosphate buffer, 3 mg of $MgSO_4$, and [^{14}C-methyl]-S-adenosylmethionine (2×10^5 cpm, 47.5 mCi/mmole). The mixture at pH 7.8 was shaken in air at 37° for 40 min and was then extracted with ether and washed with water, and the residue was reduced with $LiAlH_4$ to convert products oxidized at C-17 to the parent alcohols. The products were chromatographed on paper with the system formamide–cyclohexane[1] and two radioactive zones were obtained. These corresponded in mobility with reference samples of 2-hydroxyestradiol 2-methyl ether and 2-hydroxyestradiol 3-methyl ether.[6] The integrated radioactivity in the areas corresponded to 24,000 and 22,000 cpm, respectively, a product ratio of 1.1:1. The same incubation was repeated with another homogenate. Carrier amounts of 2-hydroxyestradiol 2-methyl ether and 2-hydroxyestradiol 3-methyl ether were added to the ether extract and the products were separated by careful column chromatography on alumina, acetylation, and recrystallization to constant specific activity. The product ratio found in this study was 1.2:1. The results are in agreement with findings of Knuppen and Breuer[1] with inactive compounds.

2-Hydroxyestradiol 3-sulfate[7] was incubated with rat liver homogenate under conditions identical with those used for the free steroid. In initial experiments the ether extracts were chromatographed and the papers were scanned for radioactivity. Four areas were detected, with mobilities corresponding to 2-methoxyestradiol, 2-methoxyestrone, 2-hydroxyestradiol 3-methyl ether, and 2-hydroxyestrone 3-methyl ether. The integrated areas of radioactivity of the two 17-hydroxy prod-

(1) R. Knuppen and H. Breuer, *Z. Physiol. Chem.*, **346**, 114 (1966).
(2) Following labeled estradiol administration to man the radioactive urinary 2-methoxyestrone region isolated by countercurrent distribution or partition chromatography was 85–90% homogeneous by reverse isotope dilution with authentic 2-methoxyestrone. Reverse isotope dilution of the same region with 2-hydroxyestrone 3-methyl ether indicated the absence of the labeled 3-methyl ether (unpublished observations from this laboratory).
(3) J. Axelrod, S. Senoh, and B. Witkop, *J. Biol. Chem.*, **233**, 697 (1958).
(4) S. Senoh, J. Daly, J. Axelrod, and B. Witkop, *J. Am. Chem. Soc.*, **81**, 6240 (1959).
(5) J. W. Daly, J. Axelrod, and B. Witkop, *J. Biol. Chem.*, **235**, 1155 (1960).
(6) J. Fishman, M. Tomasz, and R. Lehman, *J. Org. Chem.*, **25**, 585 (1960).
(7) M. Miyazaki and J. Fishman, *ibid.*, in press.