to lithium aluminum hydride reduction. However, reduction of **6** and **7** with diisobutylaluminum hydride gave the 23*R*,25-diol **10** as the sole reduction product.

To determine the absolute configuration of the 24,25-diols, we initially employed the method of Nakanishi.[14] Using $Pr(dpm)_3$ under anhydrous conditions, we obtained the desired CD spectra which varied in intensity and duration. *However, by employing the stronger chelating reagent Eu(fod)$_3$, it was possible to obtain CD spectra exhibiting very large induced split Cotton effects which were essentially unchanged over a 10-day period in reagent grade chloroform or carbon tetrachloride solvents.*[15] On the basis of the empirical rule[14] α-diols **9** and **12** were shown to possess the 24*R*-absolute configuration and α-diols **11** and **13** the 24*S*-absolute configuration. These assignments were fully confirmed by a single-crystal x-ray structural determination of diol **9**.[16]

Thus, we have developed a short and efficient construction of the 24(*R*),25-dihydroxycholesterol side chain from readily available materials. The conversion of α-diols **9** and **12** into 24(*R*),25-dihydroxycholecalciferol and 1(*S*),24(*R*),25-trihydroxycholecalciferol will be discussed in a subsequent paper.

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(12) A mixture of 103 mg (0.25 mmol) of **3**, 3 ml of dry toluene, and 2 drops of a 2% $VO(acac)_2$ solution in toluene was cooled to −78° under nitrogen. A solution of 116 mg (1.20 mmol) of 94% *tert*-butyl hydroperoxide (Pennwalt Corp.) in 1 ml of toluene was briefly dried over anhydrous sodium sulfate and was then added to the reaction mixture. The pale pink mixture was briefly stirred at −78° and was warmed to −20° and stirred for 6 h. The product was isolated with methylene chloride and this solution was washed with 10% sodium bisulfite solution and water to remove any residual oxidant. A significant loss of stereoselectivity resulted when this process was carried out at room temperature.
(13) Chromatographic separations were carried out on Merck Silica Gel 60 (70–230 mesh) with 2% acetone–methylene chloride as eluent.
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(15) The chloroform and carbon tetrachloride solvents were stored over 4 Å molecular sieves prior to use. The concentration of substrate and tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)europium(III) was 0.0002 M.
(16) The single-crystal x-ray determination was carried out by Dr. J. F. Blount and his staff at Hoffmann-La Roche Inc.

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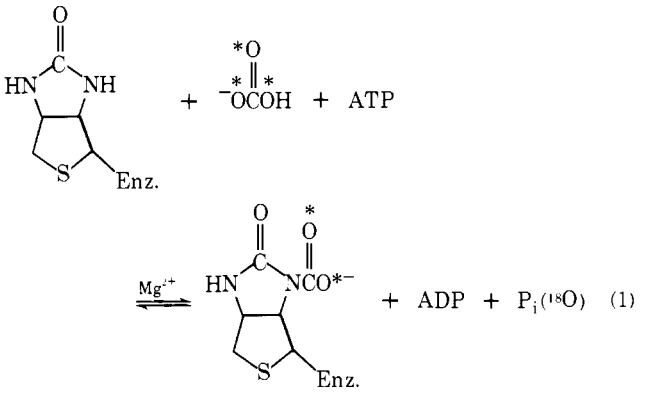
A Reaction Proceeding through Intramolecular Phosphorylation of a Urea. A Chemical Mechanism for Enzymic Carboxylation of Biotin Involving Cleavage of Adenosine 5′-Triphosphate[1]

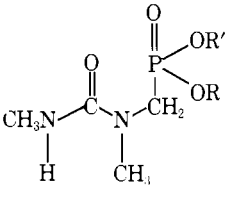
Sir:

The enzyme biotin carboxylase catalyzes the formation of *N*-carboxybiotin from biotin and bicarbonate with concomitant cleavage of ATP, leading to the ^{18}O labeling results shown in eq 1.[2,3] *N*-Carboxybiotin is the active form of coenzyme that

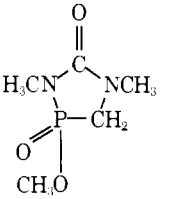


is used in further biosynthetic reactions involving fixation of carbon dioxide. Bruice's studies on the carboxylation of ureas have demonstrated that *O*-carboxylated biotin would be the expected initial nonenzymic product.[4-6] Wood has suggested[3] that the observed enzymic product[7,8] and the unlikelihood of rearrangements imply that "simple model compounds are not always reliable indicators of reactivity in the environment of an enzyme." Thus, no entirely satisfactory mechanism for carboxylation of biotin has been proposed which would also account for the labeling and ATP-cleavage results in terms of known organic reactions. We have now observed that a urea moiety is nucleophilic toward a phosphate derivative in a manner consistent with what is reported for biotin and a mechanism can be formulated in common for both sets of reactions.

We prepared **1** as a model for the reactive portions of biotin and ATP bound in the same portion of an active site by modification of the procedure of Petersen and Reuther.[9] The formaldehyde–hydrogen chloride condensation product of *N,N′*-dimethylurea[10] was dissolved in trimethyl phosphite and heated to 70° for 1 h. After removal of phosphite, chromatography on silica gel with 5% methanol in chloroform gave **2** in 28% yield (NMR($CDCl_3$): δ 3.86 (3 H, d, J = 11 Hz, P–OCH_3), 3.48 (2 H, d, J = 15 Hz, P–CH_2), 2.98 (3 H, d, J = 1 Hz, P–CH_2–N–CH_3), 2.90 (3 H, d, J = 8 Hz, P–NCH_3)). Treatment of **2** with 1 equiv of lithium hydroxide hydrate in methanol gave **1** in 83% yield. Anal. $C_5H_{12}N_2O_4PLi(CHN)$: [NMR(D_2O) δ 2.75 (3 H, s, N–CH_3), 2.98 (3 H, s, N–CH_3), 3.57 (2 H, d, J = 10 Hz, P–CH_2), 3.62 (3 H, d, J = 10 Hz, P–OCH_3)]. Neut equiv: calcd 202, found 205 ($pK_a \sim 1.3$).

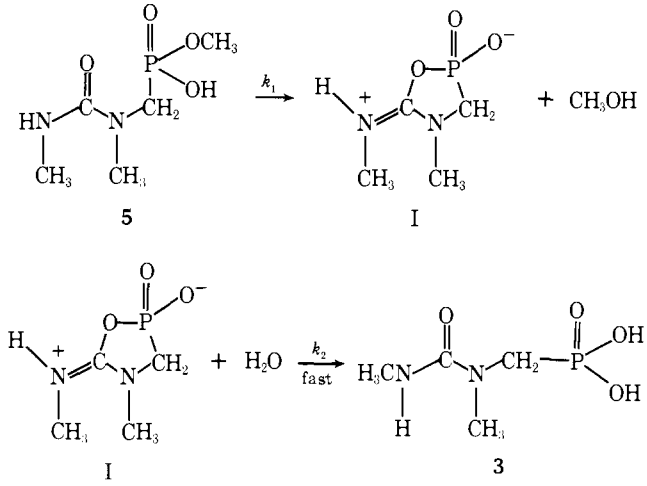


1, R = CH_3; R′ = Li
3, R = H; R′ = H
4, R = CH_3; R′ = CH_3
5, R = CH_3; R′ = H

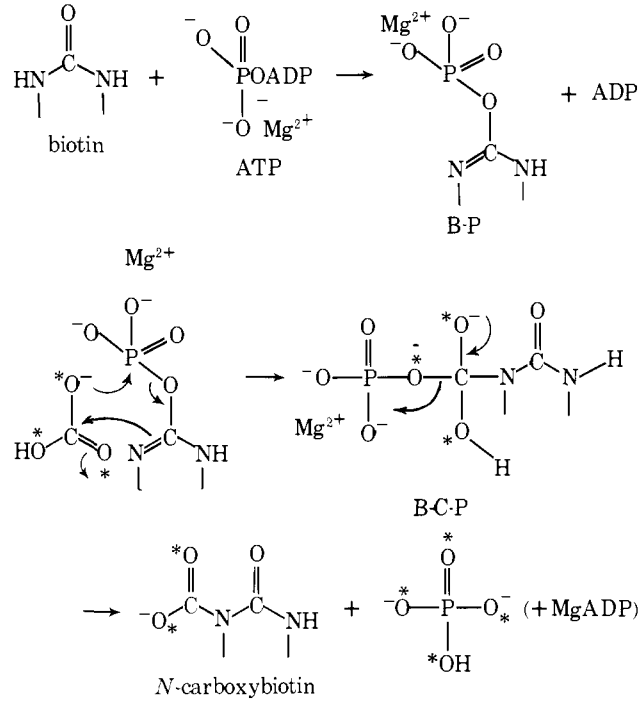


2

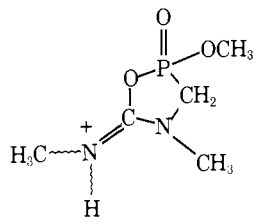
Scheme I



Scheme II



Compound **1** hydrolyzed on dissolving in dilute acid to diacid, **3**. When **3** was treated with diazomethane, diester **4** was isolated which gave satisfactory spectral and analytical (C, H, N, P) data. When **4** was treated with 1 equiv of *p*-toluenesulfonic acid in deuteriochloroform, an NMR spectrum consistent with structure **6** (δ 2.75 (3 H, s, N-CH_3), 3.00 (3 H, d, J = 1 Hz, N-CH_3), 3.75 (2 H, d, J = 11 Hz, P-CH_2); 3.78 (3 H, d, J = 11 Hz, P-OCH_3)) and 1 equiv of methanol were obtained. Addition of water to the solution of **6** converted the spectrum immediately to that of **3**, presumably via **5** as an intermediate.



6

The rate of hydrolysis of monoester **1** to diacid **3** was followed by comparing integration of the methoxyl signal of **1** and the singlet of methanol produced. Good first-order plots were obtained for the pD range 2.0–5.3 [35°; $k_{obsd} = (D^+)$ $(9.0 \times 10^{-1}\ s^{-1}\ M^{-1})$]. At lower pD's the rates were too fast to measure conveniently. The rapid hydrolysis of **1** is indicative of a reaction proceeding via intramolecular nucleophilic catalysis involving exocyclic cleavage.[11] A solution of 0.05 M dimethylurea and 0.05 M methyl methanephosphonic acid in 0.5 M DCl gave no NMR-detectable hydrolysis (<2%) when incubated at 35° for 1 month. This implies an "apparent molarity"[12] for the internal nucleophile in **1** of over 10^6 M, based on extrapolation of the observed data. These results imply that, in an active site containing a phosphate linkage and a urea bound in reactive proximity, a facile reaction is likely to occur.

In Scheme I we formulate a mechanism for the hydrolysis of **1**, consistent with observed kinetics [k_{obsd} (**1**)$(H^+) = k_1$ (**5**)] and with the restraints on structure of intermediates in phosphate ester hydrolysis.[13] In addition, the products isolated, the observation of production of **6** and the analogous reactions for urea participation in carboxylate ester reactions,[4-6] are consistent with this mechanism.

The extension of the mechanism to biotin-mediated cleavage of ATP (in the presence of bicarbonate and biotin carboxylase)[7,8] is given in Scheme II. Labeling patterns,[2,3] the observation that biotin carboxylase containing N-alkylated biotin shows phosphate-transfer activity (at a slow rate)[8] and the ability of biotin carboxylase to catalyze breakdown of carbamyl phosphate,[7,8] are consistent with this mechanism. Alternatives involving concerted reaction or free carbonyl phosphate via the *O*-phosphobiotin (B-P) intermediate are of course reasonable variations and cannot be distinguished. The chemical analogy suggested by this mechanism is that biotin is a "masked" carbodiimide dehydrating agent.[14] The *O*-phosphourea intermediate (B-P) is exactly the same type of compound proposed to occur[14-18] when dehydration to form phosphate anhydrides or esters is promoted by the conversion of carbodiimides to ureas.[19] In the case of biotin, the free energy made available by cleavage of ATP would be utilized to form the same intermediate.

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