agent was prepared from 2.40 g. 2-bromopropane-1,3-C^{14} (specific activity, 0.050 millicurie per millimole) and 0.600 g. magnesium turnings in 25 ml. anhydrous ethyl ether. The solution was cooled in an ice bath and stirred while 7.5 g. powdered anhydrous cadmium bromide (dried for 3 hr. at 120°) were added. After stirring for 30 min., the ice bath was removed and a solution of 1.50 g. triformylcholyl chloride, dissolved in 8 ml. anhydrous benzene, was added dropwise. Stirring was continued for 30 min. after the addition and the reaction mixture was heated to reflux for 1 hr. The mixture was then allowed to stand overnight. Ice water followed by sufficient 3*N* HCl solution to dissolve the precipitate was added to the reaction mixture. The benzene-ether layer was separated and washed with water until the washings were neutral. The washed solvent layer was then evaporated to dryness and the residual gum was dried *in vacuo*. This residue was saponified by heating for 1 hr. with 10 ml. 5% KOH in methanol solution. The saponification mixture was diluted with 100 ml. water and this solution was extracted with ethyl ether. The ether extract was evaporated to dryness. The residual crude ketone was subjected to chromatography on a silicic acid column. The column was washed through with benzene first and the ketone was eluted with ethyl ether–benzene (1:2). After evaporation of this eluate, 0.698 g. coprostane-3α,7α,12α-triol-24-one-27-C^{14} were obtained. Upon crystallizing twice from acetone solution, colorless crystals melting at 151–152° were obtained. This product had a specific activity of 0.051 millicurie per millimole. The infrared spectrum of this ketone exhibited a characteristic carbonyl absorption peak at 5.82 μ.

Anal. Calcd. for $C_{27}H_{46}O_4$: C, 74.60; H, 10.67. Found: C, 74.26; H, 10.63.

Coprostane-3α,7α,12α-triol-27-C^{14}. To 0.500 g. coprostane-3α,7α,12α-triol-24-one-27-C^{14} dissolved in 1 ml. ethanol, 1 ml. hydrazine hydrate (99%) was added. The mixture was swirled for a few minutes until a homogeneous solution resulted. To this solution was added 10 ml. triethylene glycol and 1 g. KOH. The mixture was heated and allowed to reflux for 30 min. The reflux condenser was then removed and the mixture heated at 180–200° for 2 hr. At the end of this period the reaction mixture was allowed to cool in a stream of nitrogen gas and it was then poured into 50 ml. of water. The precipitated compound was filtered, washed with water, and dried. The crude product was crystallized from acetone, 0.269 g. coprostane-3α,7α-12α-triol-27-C^{14} melting at 184–185° were obtained. Upon mixing with coprostane-3α,7α,12α-triol prepared by the previously cited procedure,[2] no depression in melting point was observed. The infrared spectra of the two samples were identical. Major absorption peaks were observed at 2.94 μ, 3.42 μ, 6.80 μ, 7.26 μ, 7.95 μ, 9.28 μ, 9.60 μ, 10.21 μ, 10.55 μ, 10.96 μ, and 11.70 μ.

Anal. Calcd. for $C_{27}H_{48}O_3$: C, 77.08; H, 11.50. Found: C, 77.04; H, 11.49.

Coprostane-3α,7α,12α,24ξ-tetrol-27-C^{14}. A solution containing 0.150 g. lithium aluminum hydride in 25 ml. anhydrous ethyl ether was prepared. To this solution in a flask provided with a magnetic stirrer and reflux condenser was slowly added a solution of 0.300 g. coprostane-3α,7α,12α-triol-24-one-27-C^{14} in 25 ml. anhydrous ethyl ether. Stirring was continued for 1.5 hr. at room temperature. The reaction mixture was cooled in ice and 20 ml. 2*N* H_2SO_4 solution was added slowly. The acidified reaction mixture was stirred for a few minutes and the ether layer separated and washed with water until the washings were neutral. The ether extract was then dried and evaporated to dryness. The residue was crystallized from acetone or benzene-petroleum ether to yield 0.102 g. coprostane-3α,7α,12α,24-ξ-tetrol-27-C^{14} melting at 169–170°. The infrared spectrum of this compound was qualitatively similar to that of compound I but showed an enhanced C—OH absorption peak at 2.94 μ.

Anal. Calcd. for $C_{27}H_{48}O_4$: C, 74.25; H, 11.08. Found: C, 74.14; H, 10.95.

Radiochemical purity of Compounds I, II, and III above was determined as follows: (1) A mixture of each radioactive compound and a large excess of corresponding unlabeled compound was recrystallized three times. No substantial changes (±5%) in specific activity were observed. (2) Samples of the radioactive compounds were chromatographed on paper together with samples of corresponding unlabeled compounds using the phenoxyethanol and heptane system of Neher and Wettstein[9] and the acetic acid and isopropyl ether–heptane system of Sjövall.[10] In each case practically all (90% or more) of the radioactivity was recovered from the spot corresponding to the unlabeled substance. (3) Compounds I and II were subjected to the reversed-phase partition column chromatographic procedure of Danielsson[11] modified by the use of 1:1 2-propanol–water mixture as the mobile phase. The weight and radioactivity curves of the eluted substances coincided in each case and the elution volumes corresponded to those obtained with unlabeled samples of each compound.

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Synthesis of Potential Anticancer Agents. XVIII. Analogs of Carbamoyl Phosphate[1]

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In an effort to uncover new classes of anticancer agents we have directed our attention to the synthesis of carbamoyl phosphate analogs, since carbamoyl phosphate has been shown to be involved in the *de novo* biosynthesis of pyrimidines, where it acts as the cofactor in the formation of *N*-carbamoylaspartic aicd.[2,3] The identity of this natural carbamoyl donor has been further corroborated by Hall,[4] who, in addition, has compiled a comprehensive bibliography on the subject.

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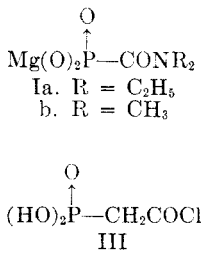
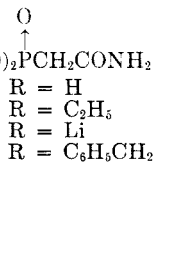


Ia. R = C_2H_5
b. R = CH_3



IIa. R = H
b. R = C_2H_5
c. R = Li
d. R = $C_6H_5CH_2$

III

In an attempt to prepare a compound which might interfere with this carbamoylation reaction, we synthesized the magnesium salt of diethylcarbamoyl phosphate (Ia). Methylcarbamoyl chloride was phosphorylated with silver dibenzyl phosphate according to the method of Zervas,[5] adapted to the preparation of mixed anhydrides of phosphoric and acetic acids by Lynen[6]; and the intermediate dibenzyl diethylcarbamoyl phosphate was catalytically hydrogenolyzed with a palladium-on-charcoal catalyst in ethanol in the presence of magnesium oxide yielding hydrated magnesium diethylcarbamoyl phosphate (Ia). Magnesium dimethylcarbamoyl phosphate (Ib), which can be obtained by a similar reaction sequence, was identified by the comparison of its infrared absorption spectrum with that of the diethyl analogy Ia; however, satisfactory elemental analyses were not obtained because of the instability of the compound, even at room temperature. There was no reaction between silver dibenzyl phosphate and diphenylcarbamoyl chloride even after prolonged refluxing of an ether suspension of the two substances.

(Carbamoylmethyl)phosphonic acid (IIa), which is sterically very similar to carbamoyl phosphate but is incapable of donating the carbamoyl group to an enzyme substrate, was also synthesized. An attempt to adapt reported procedures[7-9] for the preparation of phosphonacetic acid to the preparation of (carbamoylmethyl)phosphonic acid (IIa) was unsuccessful, since selective hydrolysis of the phosphonester group of diethyl (carbamoylmethyl)phosphonate (IIb) is not possible under the conditions described; phosphonacetic acid was obtained instead. The desired (carbamoylmethyl) phosphonic acid (IIa) was obtained by two methods: (1) Phosphonacetic acid was converted to phosphonacetyl chloride (III),[10] which upon treatment with ammonia gave the monoammonium salt of (carbamoylmethyl)phosphonic acid. The acid was purified through its dilithium salt (IIc). (2) The reaction of dibenzyl phosphite and 2-chloroacetamide according to Nylen[9] gave dibenzyl (carbamoylmethyl)phosphonate (IId) in 30% yield; catalytic hydrogenolysis of IId gave the desired (carbamoylmethyl)phosphonic acid (IIa).

Neither carbamoyl phosphate analog has shown activity against Sarcoma 180 in Swiss mice.

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(10) This acid chloride could not be purified by distillation *in vacuo*. The distillable acid chloride obtained by P. Nylen[11] from the reaction of phosphonacetic acid and phosphorus pentachloride was shown to be the trichloride.
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EXPERIMENTAL[12]

Dibenzyl diethylcarbamoyl phosphate. A solution of diethylcarbamoyl chloride (1.1 g., 7.8 mmoles) in 20 ml. of ether was added to silver dibenzyl phosphate[13] (3.3 g., 8.6 mmoles) in an atmosphere of nitrogen. The mixture was stirred with a Vibro-mixer for 3 hr. and then filtered to remove the silver salts. After evaporation of the ether *in vacuo*, the residue was heated to 80° at 8 mm. pressure to remove the last traces of unreacted diethylcarbamoyl chloride. The remaining brown oil, weighing 1.9 g. (53%), was used without further purification in the next step.

Spectral data. ν in cm.$^{-1}$ (film): 3060 (aromatic CH), 2995, 2905 (aliphatic CH); 1730 (C=O); 1640, 1505 (phenyl); 1460, 1440 (CH); 1290, 1270 (P → O); 1020, 980 (P—O—C); 740, 695 (monosubstituted benzene).

A second run yielded 5.2 g. (79%) from 6.6 g. of silver dibenzyl phosphate.

Magnesium salt of diethylcarbamoyl dihydrogen phosphate (Ia). A solution of crude dibenzyl diethylcarbamoyl phosphate (5.2 g., 14 mmoles) in 50 ml. of ethanol containing palladium catalyst on charcoal (1 g.) and magnesium oxide (0.5 g.) was shaken overnight with hydrogen in a steel bomb at 1000 p.s.i. The catalyst was removed by filtration and washed with ethanol. Two thirds of the solvent was evaporated under reduced pressure at room temperature. The addition of petroleum ether caused the precipitation of the magnesium salt, which was collected and dried *in vacuo* over phosphorus pentoxide at room temperature; yield, 2.3 g. (65%).

Spectral data. ν in cm.$^{-1}$ (KBr): 3430 (OH water); 2960 (aliphatic CH); 1660 (C=O); 1490 (CH); 1200–1070 (ionic phosphate); 980 (P—O—C).

Anal. Calcd. for $C_5H_{10}MgNO_5P.2^1/_2H_2O$: C, 22.75; H, 5.68; N, 5.30; P, 11.75. Found: C, 23.10; H, 5.99; N, 5.08; P, 11.15.

Drying at 60° *in vacuo* in an attempt to obtain anhydrous material resulted in decomposition of the molecule.

Diethyl (carbamoylmethyl)phosphonate (IIb). A mixture of 2-chloroacetamide (9.3 g., 0.1 mole) and triethyl phosphite (16.7 g., 0.1 mole) was heated at 145° for 2 hr. Unreacted 2-chloroacetamide (2 g.) was removed by vacuum distillation (1 mm.). The residue was dissolved in benzene, and the resulting solution was treated with decolorizing carbon, filtered, and then concentrated *in vacuo*. The crystalline product was removed by filtration and dried; yield, 17.4 g. (63.5%).

The analytical sample was obtained by recrystallization of the crude material once from ethyl acetate and twice from benzene (with charcoal treatment). The white crystals so obtained melted at 78–79° (lit.,[11] 78–80°). After completion of this work a recent publication has been brought to our attention [A. J. Speziale and R. C. Freeman, *J. Org. Chem.*, **23**, 1883 (1958)] describing the preparation of this compound by essentially the same procedure.

Spectral data. ν in cm.$^{-1}$ (KBr): 3360, 3160 (NH); 2972, 2915 (aliphatic CH); 1660 (amide C=O); 1625 (amide

(12) Melting points were determined on a Kofler Heizbank.
(13) Prepared by the method of Atherton *et al.*[14] except that the intermediate dibenzyl hydrogen phosphate was isolated pure.
(14) F. R. Atherton, H. T. Openshaw, and A. R. Todd, *J. Chem. Soc.*, 382 (1945).

NH); 1485, 1433, and 1378 (CH); 1240 (P → O); 1164, 1024, 975 (P—O—C).

Anal. Calcd. for $C_6H_{14}NO_4P$: N, 7.18. Found: N, 7.30.

Attempted selective hydrolysis of this compound to (carbamoylmethyl)phosphonic acid resulted in complete hydrolysis to phosphonacetic acid.

Diisopropyl (methoxycarbonylmethyl)phosphonate. Methyl bromoacetate (153 g., 1 mole) was added dropwise to hot triisopropyl phosphite (200 g., 1 mole) at a rate which maintained boiling. Distillation of the resulting solution gave a fraction boiling between 88° and 94° at 0.08–0.4 mm. This colorless liquid was redistilled, and the fraction boiling at 93–96°/0.09 mm. was collected and analyzed; yield, 172 g. (72%).

Spectral data. ν in cm.$^{-1}$ (film): 2980 (aliphatic CH); 1740 (ester C=O); 1445, 1395, 1385 (CH); 1280 (P → O); 1180, 1110, 985 (P—O—C).

Anal. Calcd. for $C_9H_{19}O_5P$: C, 45.38; H, 8.04; P, 13.01. Found: C, 45.78; H, 8.75; P, 12.58.

Phosphonacetic acid. Diisopropyl (methoxycarbonylmethyl)phosphonate (72 g., 0.3 mole) was heated with concentrated hydrochloric acid (300 ml.) on a water bath for 4 hr. A portion of the hydrochloric acid (150 ml.) was removed by distillation at atmospheric pressure, and the remainder was removed under reduced pressure. Distillation with benzene removed the last traces of water azeotropically. Evaporation of the benzene gave an oil which crystallized on standing overnight in a desiccator over phosphorus pentoxide and potassium hydroxide. Recrystallization from acetic acid gave 33.2 g. (79%) of the free acid, m.p. 143° (lit.[8] m.p. 142–143°).

Spectral data. ν in cm.$^{-1}$ (KBr): 2900 (acidic hydrogen); 2300 (shoulder, P—OH); 1705 (acid C=O); 1410 (CH); 1125 (P → O, hydrogen bonded).

Anal. Calcd. for $C_2H_5O_5P$: C, 17.16; H, 3.60; P, 22.13. Found: C, 17.43; H, 3.43; P, 21.78.

Dibenzyl (carbamoylmethyl)phosphonate (IId). Molten sodium (2.3 g., 0.1 mole) was shaken with a Vibro-mixer in boiling toluene (50 ml.), and the fine dispersion was allowed to cool with shaking to give powdered sodium. The toluene was decanted and replaced with ether (200 ml.). Absolute ethanol (4.6 g., 0.1 mole) was added and the suspension was refluxed until all the sodium had disappeared (8 hr.). A solution of dibenzyl phosphite (26.3 g., 0.1 mole) in 50 ml. of ether was added dropwise in an atmosphere of nitrogen to give a clear solution of sodium dibenzyl phosphite. This solution was added dropwise to a suspension of 2-chloroacetamide (9.3 g., 0.1 mole) in ether with stirring and cooling. After the addition was completed, the mixture was heated under reflux for 1 hr. The ether was decanted from the gummy precipitate which had formed, and was washed with water (3 × 100 ml.), dried over magnesium sulfate, and evaporated under reduced pressure. The residue (3.6 g.) from the ether evaporation crystallized when the walls of the vessel containing it were scratched. Recrystallization of a sample of this material from water gave long, colorless needles, m.p. 93°.

The gummy residue from the ether decantation was dissolved in benzene and washed with water (3 × 100 ml.) to remove sodium chloride. Evaporation of the benzene solution, which had been dried over magnesium sulfate, gave a residue (5.8 g.) which crystallized when scratched. After recrystallization from water, the product melted at 93–94°; total yield, 9.4 g. (29%).

Spectral data. ν in cm.$^{-1}$ (KBr): 3360, 3180 (NH); 3050 (aromatic CH); 2980, 2905 (aliphatic CH); 1665 (amide C=O); 1645 (amide); 1505 (phenyl); 1430, 1410, 1390 (CH); 1240 (P → O); 1005, 980 (P—O—C); 750, 700 (monosubstituted benzene).

Anal. Calcd. for $C_{16}H_{18}NO_4P$: C, 60.19; H, 5.68; N, 4.39; P, 9.70. Found: C, 60.67; H, 5.46; N, 4.32; P, 9.75.

(Carbamoylmethyl)phosphonic acid (IIa) (*A*). *Free acid.* A solution of dibenzyl (carbamoylmethyl)phosphonate (1 g., 3 mmoles) in 25 ml. of absolute ethanol was hydrogenated in the presence of 5% palladium-on-charcoal catalyst (0.5 g.). Hydrogen uptake stopped after 165 ml. had been consumed (calculated 155 ml.). The catalyst was removed by filtration and washed with ethanol. The residue from the evaporation of the combined filtrate and washings was recrystallized from methanol; yield of white needles, 0.3 g. (68%); m.p. 171°.

Spectral data. ν in cm.$^{-1}$ (KBr): 3375, 3220 (NH); 2400–2300 (P—OH); 1675 (amide C=O); 1605 (amide NH); 1460, 1405 (CH); 1170 (P → O, hydrogen bonded).

Anal. Calcd. for $C_2H_6NO_4P$: C, 17.27; H, 4.35; N, 10.08; P, 22.28. Found: C, 17.58; H, 4.42; N, 10.22; P, 22.41.

B. Dilithium salt (IIc). Phosphonacetic acid (14 g., 0.1 mole) and 70 g. of thionyl chloride were mixed. Immediate evolution of hydrogen chloride occurred. When the evolution began to slow down, heat was applied. After completion of the reaction, excess thionyl chloride was removed under reduced pressure with the addition of several portions of benzene. The residue, a reddish yellow oil free of thionyl chloride odor, was dissolved in dry dioxane (180 ml.), and gaseous ammonia was bubbled through the solution for 1 hr. The yellow precipitate that formed was filtered off rapidly and extracted with hot glacial acetic acid. Most of the ammonium chloride remained undissolved, but a small amount crystallized from the acetic acid on cooling. It was removed by filtration, and the acetic acid was evaporated under reduced pressure. The remaining orange-colored oil was dissolved in 200 ml. of 5% hydrochloric acid. Neutralization with a saturated solution of lithium hydroxide, followed by the addition of an equal volume of ethanol, gave an almost white precipitate, which was collected by filtration and dried *in vacuo* over phosphorus pentoxide at 110°.

Spectral data. ν in cm.$^{-1}$ (KBr): 3200 (NH); 1660 (amide C=O); 1610 (amide NH); 1440, 1390 (CH); 1105, 1085 (ionic phosphonate); 1005 (P—C).

Anal. Calcd. for $C_2H_4Li_2NO_4P$: C, 15.92; H, 2.67; N, 9.29; P, 20.53. Found: C, 16.24; H, 3.02; N, 9.39; P, 20.36.

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An Extension of the Woodward Rules Concerning Alkyl Substituents in Conjugated Aliphatic Systems

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It is known[1a,b] that alkyl substitution in α,β-unsaturated aldehydes and ketones affords ap-

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