listed in Table I have about the same rotational freedom for the phenyl, slightly less than that in III and IV, and the chemical shifts of the phenyl protons fall in a relatively narrow range, somewhat below III and IV. In addition to the results reported in Table I, we have obtained spectra of solutions of oils isolated from the mother liquors resulting from filtration of I and II from their crude reaction mixtures. Evidence has been presented[1] that these mother liquors are rich in the *cis*-4-phenyl-3,5-dicarboalkoxy-2-pyrazolines. In these spectra there are resonances reported for either compound I or II as well as additional bands. The latter are believed to be characteristic of the *cis* isomers, since the phenyl peaks fall at lower fields, one chemical shift being negative. In the *cis* isomer, the phenyl group is wedged between the two substituents. The shift of the resonance of the phenyl group to lower magnetic field is therefore attributed to steric effects, very probably a direct "repulsive unshielding" of the phenyl protons.[9]

GAINESVILLE, FLA.

(9) G. V. D. Tiers, *J. Am. Chem. Soc.*, **78**, 2914 (1956).

[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACOLOGY, YALE UNIVERSITY SCHOOL OF MEDICINE]

Potential Deoxyribonucleic Acid Cross-linking Agents. 8,8′-Bispurines[1,2]

HENRY G. MAUTNER

Received September 28, 1960

As part of a program involving the preparation of compounds, incorporation of which could result in interhelical cross-linking of deoxyribonucleic acid, a group of 8,8′-bispurines connected by a four-carbon chain has been synthesized. 8,8′-Bisdihydropurinyl was obtained by the reaction of 5,6-diaminopyrimidine with glyoxal.

The synthesis of 8,8′-bispurines was undertaken as part of a program aimed at preparing compounds specifically capable of cross-linking deoxyribonucleic acid. Deoxyribonucleic acid is now generally accepted as having the structure of a double helix,[3] the twin strands of which are held together by hydrogen-bonding between adenine and thymine or between guanine and cytosine. This interaction involves groups attached to the 2- and 4-positions of the pyrimidines and the 2- and 6-positions of the purines.

It is possible to consider either intra-helical or extra-helical cross-linking of deoxyribonucleic acid. In the former case, the groups responsible for the hydrogen-bonding between purine and pyrimidine rings could be so modified as to lead to unusually strong interaction within the double structure inhibiting its replication. It seems possible that 6-thioguanine, which is incorporated into deoxyribonucleic acid,[4] exerts its carcinostatic effects by forming stronger hydrogen bonds through its highly polarized C–S group with cytosine than does guanine. Inter-helical cross-linking would imply interaction between adjacent double helices. The antitumor activity of alkylating agents, which has been postulated[5] as being due to cross-linking between the phosphate groups located on the outer shell of deoxyribonucleic acid, might provide an example of possible inter-chain interaction.

Attempts have been made to synthesize compounds the incorporation of which could result in specific cross-linking between adjoining molecules of deoxyribonucleic acid.[6]

In one approach to this problem a group of 8-purinyl nitrogen mustards has been synthesized,[7] in another approach it was decided to prepare bispurines. Double incorporation of these—or of their ribonucleosides or deoxyribonucleosides—into deoxyribonucleic acid would result in cross-linking of the inter-helical type.

The 8-position was chosen for the attachment of the rather bulky groups being introduced here, since there is good evidence,[8] that hydrogen-bonding between purines and pyrimidines is necessary for deoxyribonucleic acid synthesis. Accordingly, for incorporation of the bis-compounds to take place it would seem desirable for the cross-linking groups to extend radially toward the periphery of the double helix without interfering with intra-helical hydrogen-bonding. For this purpose the 8-position of purines and the 5-position of cytosine or the methyl group of thymine would seem to be the most suitable points of attachment.

As a first approach to the synthesis of 8,8′-bispurines, the condensation of 5,6-diaminopyrimi-

(1) Part of this material was presented at the meeting of the Medicinal Chemistry Section of the American Chemical Society, Cleveland, Ohio, April, 1960, 9-N.

(2) This work was supported, in part, by a grant (CY-3937) from the National Institutes of Health, Public Health Service.

(3) J. D. Watson and F. H. C. Crick, *Nature*, **171**, 737 (1953).

(4) G. A. LePage, *Cancer Res.*, 20, 403 (1960).

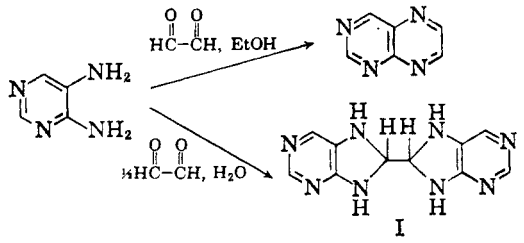
(5) K. A. Stacey *et al.*, *Ann. N.Y. Acad. Sci.*, **68**, 682 (1958).

(6) H. G. Mautner, *Yale J. Biol. Med.*, **32**, 323 (1960).

(7) S. H. Chu, J. E. Harris, and H. G. Mautner, *J. Org. Chem.*, **25**, 1759 (1960).

(8) A. Kornberg, *Science*, **131**, 1503 (1960).

dine, with glyoxal which ordinarily yields pteridine,[9] was reexamined. It was found that when two moles of the pyrimidine were permitted to react with one mole of glyoxal in concentrated aqueous solution, 8,8′-bisdihydropurinyl(I) was obtained: A similar



I

reaction, using 5-amino-6-methylaminopyrimidine, had been observed by Fidler and Wood.[10]

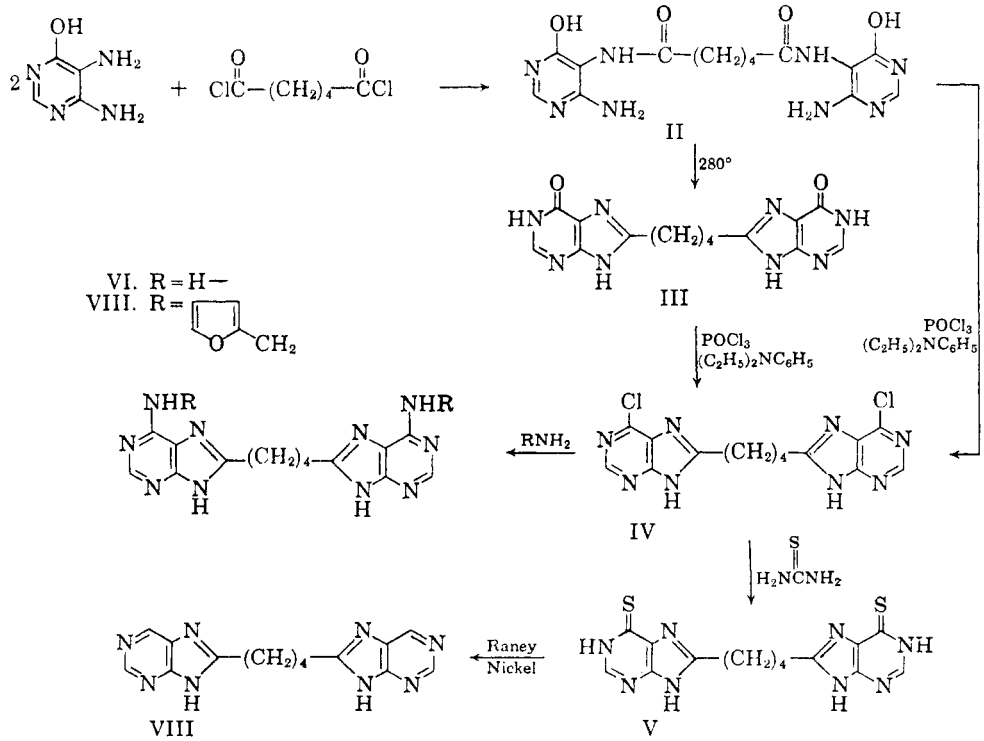
The reaction of 4-amino-, 4-hydroxy-, and 4-thio-5,6-diaminopyrimidine with glyoxal yielded only 4-substituted pteridines, whatever the reaction conditions, perhaps because substitution in position 4 lowers the water solubility of 5,6-diaminopyrimidine and that perforce more dilute solutions had to be used in which intra-molecular condensation would be favored. Attempts to increase the likelihood of inter-molecular reaction by using α,ω-dialdehydes of greater chain length, such as glutaraldehyde or terephthaldehyde, have not been successful. The following reaction sequence was then undertaken:

it seemed likely that bisamides would result from the use of dibasic acid chlorides. The reaction of 4-hydroxy-5,6-diaminopyrimidine with adipyl chloride led to the quantitative formation of *N,N′*-bis - 5 - (4 - hydroxy - 6 - aminopyrimidyl)adipamide (II), even though the corresponding reaction using succinyl chloride was unsuccessful.

Fusion of the sodium salt of the amide (II) at 280° resulted in double dehydration, 1,4-bis-8-(6-hydroxypurinyl)butane (III) being obtained in 25% yield. Reaction of the bishypoxanthine (III) with phosphorus pentasulfide in tetralin resulted only in partial conversion to the dimercapto compound (V). Only unpurifiable decomposition products were obtained when this reaction was repeated in pyridine, which is ordinarily an excellent solvent for thiations.[12]

Reaction of the dihydroxy compound (III) with phosphoryl chloride and diethylaniline, in a manner similar to that used in the formation of 6-chloropurine from hypoxanthine,[13] gave a poor yield (5–23%) of 1,4-bis-8-(6-chloropurinyl)butane (IV).

Since the bischloropurine (IV) was an important intermediate in the reaction sequence described here, it was attempted to circumvent the very meager over-all yield of the successive dehydration and chlorination reactions, by attempting to prepare the chloro compound in one step from the bisamide (II). Phosphoryl chloride alone, which had



As acid chlorides react with 5,6-diaminopyrimidines to form 5-amino-6-amido compounds,[11] been used to prepare 6-chloro-8-arylpurines from 4-hydroxy-5-arylamido-6-aminopyrimidines,[14] was

(9) A. Albert, D. J. Brown, and G. Cheeseman, *J. Chem. Soc.*, 483 (1951).

(10) W. E. Fidler and H. C. S. Wood, *J. Chem. Soc.*, 331 (1956).

not useful in the formation of the desired product. However, the combination of phosphoryl chloride and diethylaniline caused the simultaneous dehydration and chlorination of the bisamide II to the bischloropurine IV.

1,4-Bis-8-(6-chloropurinyl)butane (IV) could be converted quantitatively to the dithio compound (V) by the use of thiourea[13] in ethanol. Use of Raney nickel resulted in the desulfurization of bismercaptopurine (V) to 1,4-bis(8-purinyl)butane (VIII). Treatment with selenourea did not convert the bischloro- to the bisseleno compound, even though the analogous reaction with 6-chloropurine had yielded 6-selenopurine.[15]

Treatment of the dichloro compound (IV) with alcoholic ammonia in a bomb yielded 1,4-bis-8-(6-aminopurinyl)butane (VI). Since 6-furfurylaminopurine is a potent plant growth promoter,[16] it seemed of interest to prepare the corresponding bis-compound (VII). This was accomplished by the reaction of the bischloro compound (IV) with furfurylamine in refluxing Methyl Cellosolve.

Most of the compounds prepared lacked sharp melting points and decomposed over wide ranges at very high temperatures; thus, purification procedures had to be followed by measurements of ultraviolet absorption. In all cases the spectra of the biscompounds described and of the corresponding monomeric purines were very similar.

Studies of the synthesis of bispurines linked by longer alkyl chains, of bispyrimidines, and of the ribonucleosides of the above compounds are in progress.

EXPERIMENTAL

8,8'-Bisdihydropurinyl (I). A solution of 2.0 g. (0.0182 mole) of 5,6-diaminopyrimidine in 10 ml. of water was treated with 0.69 g. (0.0091 mole) of glyoxal monohydrate[17] (British Drug Houses) and the mixture heated on a steam bath for 5 min. On cooling, a voluminous mass of delicate, white needles weighing 0.7 g. (32%) separated. The product was washed with ethanol and recrystallized from 50% ethanol; m.p. 256°.

Anal.[18] Calcd. for $C_{10}H_{10}N_8$: C, 49.58; H, 4.16; N, 46.26. Found: C, 49.48; H, 4.39; N, 46.20.

Ultraviolet spectrum. *p*H 3: λ_{max} 304 mμ, ϵ_{max} 17,700. *p*H 7: λ_{max} 255 mμ, ϵ_{max} 9,310; λ_{max} 305 mμ, ϵ_{max} 15,500. *p*H 10: λ_{max} 255 mμ, ϵ_{max} 9,200; λ_{max} 307 mμ; ϵ_{max} 16,560.

N,N'-Bis-5-(4-hydroxy-6-aminopyrimidyl)adipamide (II). A solution of 5.04 g. (0.04 mole) of 4-hydroxy-5,6-diaminopyrimidine in 160 ml. of 1*N* sodium hydroxide was chilled in an ice bath. With vigorous stirring and continued cooling, adipyl chloride, 3.64 g. (0.02 mole), was added in small portions. When all the acid chloride had reacted the solution was acidified with acetic acid. The faintly cream-colored product was removed by filtration, washed with water and dried. A yield of 6.7 g. (93%) was obtained. The analytical sample was recrystallized twice from boiling water.

Anal. Calcd. for $C_{14}H_{18}N_8O_4$: C, 46.40; H, 5.01; N, 30.93. Found; C, 46.35; H, 5.21; N, 30.62.

Ultraviolet spectrum. *p*H 10: λ_{max} 261 mμ, ϵ_{max} 9,160.

1,4-Bis-8-(6-hydroxypurinyl)butane (III). A suspension of 5.0 g. (0.0138 mole) of *N,N'*-bis-5-(4-hydroxy-6-aminopyrimidyl)adipamide in 4.58 ml. of 6*N* sodium hydroxide was evaporated to dryness on a steam bath under aspirator suction. The mixture was then heated for 2 hr. at 280° in a bath of Wood's metal. The residue was dissolved in 100 ml. of boiling water, treated with decolorizing carbon and filtered. The hot, clear, light red filtrate was acidified with 4 ml. of glacial acetic acid and filtered immediately. A yield of 1.30 g. (27.4%) of light yellow product was obtained. The analytical sample was prepared by dissolving a portion of the product in hot 1% sodium carbonate and subsequent acidification of the solution. Water of hydration was lost when the compound was heated in a drying pistol over boiling *p*-xylene and rapidly regained on exposure to the atmosphere.

Anal. Calcd. for $C_{14}H_{14}N_8O_2.H_2O$: C, 48.83; H, 4.68; N, 32.54. Found: C, 48.75; H, 5.01; N, 32.77.

Ultraviolet spectrum. *p*H 10: λ_{max} 260 mμ, ϵ_{max} 21,700.

1,4-Bis-8-(6-chloropurinyl)butane (IV). *Method a.* A mixture of 1.0 g. (0.00291 mole) of 1,4-bis-8-(6-hydroxypurinyl)-butane, 2.5 ml. of *N,N*-diethylaniline, and 30 ml. of phosphoryl chloride was heated to reflux for 6 hr. After standing overnight at room temperature, the dark solution was evaporated under suction. The residual syrup was poured over 50 g. of crushed ice. The mixture was made strongly alkaline with 10*N* sodium hydroxide and stirred until all the heavy, dark oil had dissolved. The solution was extracted three times with ice cold ether. The aqueous layer was taken to a *p*H of 2 with concentrated hydrochloric acid and filtered immediately to remove the black tar which separated. The yellow filtrate was extracted with ether for 24 hr. in a liquid-liquid extractor. A yield of 0.25 g. (23%) of coarse yellow needles separated. The product was purified by being dissolved in 3% sodium carbonate, treated with Norit, filtered, and reprecipitated with acetic acid.

Anal. Calcd. for $C_{14}H_{12}N_8Cl_2.1/2\ H_2O$: C, 45.17; H, 3.52; N, 30.11. Found: C, 45.32; H, 3.64; N, 29.98.

Ultraviolet spectrum. *p*H 10: λ_{max} 279 mμ, ϵ_{max} 23,800.

Method b. A mixture of 2.0 g. of *N,N'*-bis-5-(4-hydroxy-6-aminopyrimidyl)adipamide, 5 ml. of *N,N*-diethylaniline and 50 ml. of phosphoryl chloride was heated to reflux for 6 hr. The material was worked up as described above. A yield of 0.28 g. (13%) was obtained.

1,4-Bis-8-(6-mercaptopurinyl)butane (V). A mixture of 0.3 g. of bischloropurine (IV) and 3.0 g. of thiourea was heated to reflux in 150 ml. of absolute ethanol for 90 min. After cooling, the light orange product was removed by filtration, washed with water, and then with ethanol. A yield of 0.3 g. (100%) was obtained. Attempts to purify the material through recrystallization or base-acid precipitation resulted in a decrease of purity, as judged by spectroscopic data.

Anal. Calcd. for $C_{14}H_{14}N_8S_2H_2O$: C, 44.66; H, 4.28; N, 29.77. Found: C, 45.03; H, 4.18; N, 29.70.

Ultraviolet spectrum. *p*H 10: λ_{max} 233 mμ, ϵ_{max} 26,750; λ_{max} 311 mμ, ϵ_{max} 32,400.

1,4-Bis-8-(6-aminopurinyl)butane (VI). An ice cold solution of 0.15 g. (0.000413 mole) of bischloropurine (IV) in 30 ml. of absolute ethanol was saturated with ammonia gas and then sealed in a bomb which was heated to 150° for 6

(11) W. Wilson, *J. Chem. Soc.*, 1157 (1948).

(12) G. B. Elion and G. H. Hitchings, *J. Am. Chem. Soc.*, **77**, 1676 (1955); E. Klingsberg and D. Papa, *J. Am. Chem. Soc.*, **73**, 4988 (1951).

(13) A. Bendich, P. J. Russel, and J. J. Fox, *J. Am. Chem. Soc.*, **76**, 6073 (1954).

(14) G. B. Elion, E. Burgi, and G. H. Hitchings, *J. Am. Chem. Soc.*, **73**, 5235 (1951).

(15) H. G. Mautner, *J. Am. Chem. Soc.*, **78**, 5292 (1956).

(16) C. O. Miller *et al.*, *J. Am. Chem. Soc.*, **77**, 2262 (1955).

(17) We are indebted to Professor Adrien Albert of the Australian National University for suggesting the use of this crystalline polymer instead of glyoxal solution.

(18) Analyses were carried out at Midwest Microlab Inc., Indianapolis, Ind.

hr. On cooling, 0.1 g. (75%) of faintly tan needles separated. The product was dissolved in 50 ml. of 0.01*N* hydrochloric acid. Addition of 2 ml. of concd. ammonium hydroxide to the hot solution resulted in the separation of white needles.

Anal. Calcd. for $C_{14}H_{16}N_{10}$: C, 51.84; H, 4.97; N, 43.19. Found: C, 51.76; H, 5.08; N, 43.16.

Ultraviolet spectrum. 0.1 *N* hydrochloric acid: λ_{max} 267 mμ, ϵ_{max} 29,500.

1,4-Bis-8-(6-furfurylaminopurinyl)butane. A mixture of 0.1 g. (0.000269 mole) of bischloropurine (IV), 1 ml. of furfurylamine, and 20 ml. of ethylene glycol monomethyl ether was heated to reflux for 4 hr. After standing overnight at room temperature, the solution was reduced in volume to 4 ml. under aspirator suction at 60°. Addition of 4 ml. of absolute ethanol resulted in the separation of 0.08 g. (60%) of tan crystals. Recrystallization from ethanol-Methyl Cellosolve (1:1) yielded pale yellow needles.

Anal. Calcd. for $C_{24}H_{24}N_{10}O_2$: C, 59.49; H, 4.99; N, 28.91. Found: C, 59.15; H, 5.32; N, 28.61.

Ultraviolet spectrum. 0.1 *N* hydrochloric acid: λ_{max} 279 mμ, ϵ_{max} 38,700.

1,4-Bis(8-purinyl)butane (VIII). A suspension of 0.2 g. (0.000532 mole) of bismercaptopurine (V) in 30 ml. of 10% ammonium hydroxide was heated to boiling and treated with 0.8 g. of ethanol-wet Raney nickel. The mixture was permitted to reflux for 2 hr. and filtered immediately. On standing, 0.03 g. (19.5%) of yellowish white crystals separated from the colorless solution. The products was recrystallized from boiling water.

Anal. Calcd. for $C_{14}H_{14}N_8$: C, 57.13; H, 4.79; N, 38.08. Found: C, 57.20; H, 5.10; N, 38.40.

Ultraviolet spectrum. *p*H 10: λ_{max} 272 mμ, ϵ_{max} 19,200.

Acknowledgment. In the synthetic work described, Miss Elaine Perry gave valuable and patient assistance.

NEW HAVEN, CONN.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF MICHIGAN]

Synthesis of Potential Anticancer Agents. VII. *N,N*-Ethyleneureido Analogs of Some Amino Acids[1,2]

ROBERT C. ELDERFIELD AND R. STANLEY McELHINNEY

Received August 5, 1960

The present status in cancer chemotherapy of amino acid analogs containing alkylating groups has been reviewed. Synthesis of isocyanato esters of five selected analogs and incorporation of ethylenimine functions are described.

Many of the early attempts to design anticancer agents led to compounds which could be broadly classified as either alkylating agents or antimetabolites. More recently there has been an increasing tendency to combine these functional features in the same molecule and, as a result, a number of biologically familiar substances, such as pyrimidines, sugars, amino acids, and even steroids, have been modified by incorporation of β,β'-bischloroethylamino (nitrogen mustard), ethylenimino, methanesulfonate, and other alkylating functions. An alternative conception of the carcinolytic activity observed with these newer compounds is based on the "carrier" hypothesis, which pictures the alkylating function as being transported by the familiar part of the molecule to the body site where the latter is normally metabolized. Baker[3] has pointed out that, for naturally occurring substances, *e.g.*, L-phenylalanine, the carrier and antimetabolite concepts are one and the same, since the *carrier* moiety in L-phenylalanine mustard fits the enzymatic site for the *metabolite*.

The field of amino acids and peptides with antimetabolic activity has been discussed in a recent symposium[4] which laid strong emphasis on the cytotoxic properties of some of these compounds.

Of the amino acid-alkylating function combinations prepared to date, the majority are nitrogen mustards[5] in which the mustard function occurs at a "remote" part of the molecule[3,6] or replaces the α-amino group.[7] Its incorporation as an amide[8] and as a urethan is also under investigation.[9] A few other alkylating functions when incorporated into an amino acid have known antitumor activity or are being evaluated, e.g., the diazoacetyl,[10-12] fluoroacetyl[3] and diazonium[3] substituents. On the

(1) The work presented was done under Research Grant CY-2961 from the National Cancer Institute to the University of Michigan.

(2) For Paper VI in this series, see W. R. Vaughan, R. S. Klonowski, *J. Org. Chem.*, **26**, 145 (1961).

(3) H. F. Gram, C. W. Mosher, and B. R. Baker, *J. Am. Chem. Soc.*, **81**, 3103 (1959).

(4) *Amino Acids and Peptides with Antimetabolic Activity*, G. E. W. Wolstenholme and C. M. O'Conner, eds., Churchill, London, 1958.

(5) F. H. Bergel, *Ann. N.Y. Acad. Sci.*, **68**, 1238 (1958).

(6)(a) H. E. Smith and J. M. Luck, *J. Org. Chem.*, **23**, 837 (1958); (b) G. E. McCasland, R. Horvat, J. Korntvedt, and A. Furst, *J. Org. Chem.*, **23**, 1568 (1958); (c) T. S. Osdene, D. N. Ward, W. H. Chapman, and H. Rakoff, *J. Am. Chem. Soc.*, **81**, 3100 (1959); (d) F. H. Bergel, J. M. Johnson, and J. A. Stock, *Chem. & Ind. (London)*, 1487 (1959).

(7)(a) M. Izumi, *Pharm. Bull. (Japan)*, **2**, 275, 279 (1954); *J. Am. Pharm. Assoc.*, **44**, 132 (1955); W. L. Nyhan and H. Busch, *Chem. Eng. News*, May 5, 1958, p. 46.

(8) O. M. Friedman and R. Chatterji, *J. Am. Chem. Soc.*, **81**, 3750 (1959).

(9) R. B. Ross, *J. Chem. Ed.*, **36**, 370 (1959).

(10) H. A. de Wald and A. M. Moore, *J. Am. Chem. Soc.*, **80**, 3941 (1958); F. Weygand, H. J. Bestmann, and E. Klieger, *Chem. Ber.*, **91**, 1037 (1958).

(11) Y. Liwschitz, R. D. Irsay, and A. I. Vincze, *J. Chem. Soc.*, 1308 (1959).

(12) B. R. Baker, *Biochem. Pharmacol.*, **2**, 1037 (1959).