COMMUNICATIONS TO THE EDITOR

RELATIONSHIP OF THE FOLINIC ACID GROUP AND THE LEUCONOSTOC CITROVORUM FACTORS

Sir:

The reported nutritional requirement of *Leuconostoc citrovorum* 8081 for thymidine[1] or a factor(s) in refined liver extracts[2] suggested the possibility that the organism required an antipernicious anemia principle. In the isolation of thymidine,[3] this factor and erythrotin,[4] a member of the vitamin B_{12} group and probably identical with vitamin B_{12}, were separated quantitatively. While neither of these fractions possess the activity of the original liver extract for *Leuconostoc citrovorum* in a previously described medium[5] supplemented with pyridoxine, the two fractions on recombination possessed the full activity. The thymidine containing fraction could be replaced by 0.03–0.1 γ per cc. of crystalline thymidine while the erythrotin-containing fraction could not be replaced by crystalline erythrotin. However, concentrates of the folinic acid[6] were found to be highly effective in replacing the erythrotin fraction, and the relative potencies of these fractions determined by *Lactobacillus casei* test and this modified test parallelled closely.

A concentrate of folinic acid 200,000 times as active as an enzymatic digest of liver in the *Lactobacillus casei* test, elicited a half-maximal response in the *Leuconostoc citrovorum* test at 0.0001–0.0002 γ per cc. In the absence of thymidine, 0.001 γ per cc. of this factor was required for the same response. Thymidine at a concentration of 10–20 γ per cc. can also replace the folinic acid group; consequently, the synergistic action of the folinic acid group and thymidine in stimulating the growth of the organism resulted in the high activity of purified liver extracts which we have previously found to contain large amounts of thymidine.[3]

Other factors associated with the folinic acid appear to be effective for this organism, but folic acid is essentially inactive under our testing conditions. Very mild acid hydrolysis destroys folinic acid but forms a compound with biological activities corresponding to folic acid.

This synergistic action and interchangeability of thymidine and the folinic acid group indicate the functioning of this group in the biosynthesis of thymidine as well as further involvement of thymidine concerning the biosynthesis of the active coenzyme form of folinic acid for this organism.

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(1) Snell, *et al.*, *J. Biol. Chem.*, **175**, 473 (1948).
(2) Sauberlich and Baumann, *ibid.*, **176**, 165 (1948).
(3) Shive, *et al.*, THIS JOURNAL, **70**, 2299 (1948).
(4) Shive, *Ann. New York Acad. Science*, in press, presented before the New York Acad. Science, Feb., 1949.
(5) Snell, *et al.*, *J. Biol. Chem.*, **143**, 519 (1942).
(6) Bond, *et al.*, THIS JOURNAL, **71**, 3852 (1949).

THE FOLINIC ACID GROUP, A SERIES OF NEW VITAMINS RELATED TO FOLIC ACID

Sir:

By application of *inhibition analysis* to a study of factors functionally related to *p*-aminobenzoic and folic acids, testing procedures for a wide variety of new factors occurring in refined liver extracts have been developed.[1,2] One of these methods developed about two years ago, involved the prevention of the toxicity of methylfolic acid for *Lactobacillus casei* in a previously described medium[3] supplemented with thymine, purines, folic acid (0.001 γ per cc.) and methylfolic acid (1 γ per cc.). Under these conditions, only the folic acid group was known to prevent competitively the toxicity of methylfolic acid. However, liver extracts, both crude and refined, which prevent the toxicity in a competitive manner, are approximately 15 times as active as can be accounted for on the basis of their folic acid content determined by assay with *Lactobacillus casei* in the absence of the inhibitor. A similar technique has been employed with *Streptococcus faecalis* R in demonstrating an unusual activity for formylfolic acid.[4]

With the aid of this assay based on this differential in activity, one of the active principles has been concentrated more than 200,000 fold from enzymatic digests of hog liver. A half-maximal response of *Lactobacillus casei* is obtained in the presence of 0.002 γ per cc. of the concentrate which is somewhat more active than folic acid under these testing conditions. Depending upon the time of incubation and response at which the comparison is made, the concentrate is from 10 to 100 times as active as folic acid in preventing the toxicity of methylfolic acid (1 γ per cc.) for *Streptococcus faecalis* R. On the basis of structure and functional relationship to folic acid, this active principle has been termed folinic acid. On the basis of estimated purity of the concentrate, folinic acid does not appear to be less active than folic acid in promoting the growth of either organism in the absence of the inhibitor.

(1) Shive, *et al.*, THIS JOURNAL, **70**, 2299 (1948).
(2) Shive, *Ann. New York Acad. Science*, in press, presented before the New York Acad. of Science, Feb., 1949.
(3) Rogers and Shive, *J. Biol. Chem.*, **172**, 100, 751 (1947).
(4) Gordon, *et al.*, THIS JOURNAL, **70**, 878 (1948).

While folinic acid accounts for the major portion of the activity of extracts of hog liver, another factor with similar physical and biological properties occurs in these extracts. At least two other substances possessing activity in the assay have been detected by means of paper chromatography. Consequently, it appears that a group of compounds, the folinic acid group, possess activity similar to that of folinic acid.

Since the folinic acid group is utilized more effectively than folic acid for several organisms, the possibility exists that it may be more active than folic acid in the treatment of sprue, nutritional and pernicious anemia, and other nutritional deficiencies related to the folic acid and vitamin B_{12} groups.

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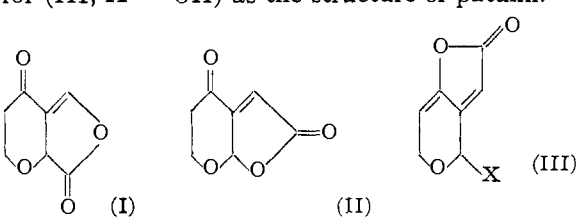
THE STRUCTURE OF PATULIN

Sir:

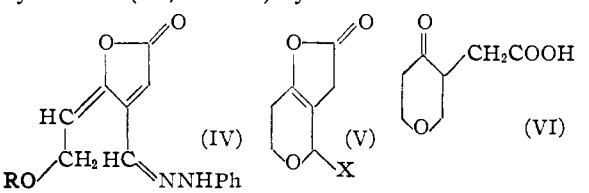
Recent evidence has required revision of the accepted structure (I)[1] of the antibiotic mold metabolite, patulin, and two new formulations, (II)[2] and (III, X = OH),[3] have been advanced. The following data now afford additional strong support for (III, X = OH) as the structure of patulin.



(I) (II) (III)

Structure (III, X = OH) possesses three structural characteristics: free hydroxyl group, lactal ring and doubly-unsaturated lactone system. Presence of a free O–H band (2.73μ), in the infrared spectrum of patulin and its absence in patulyl acetate (III, X = OAc) and in patulyl chloride (III, X = Cl), retention of the characteristic double bond ultraviolet and infrared spectra of patulin in these derivatives (Patulin: u.v., 275 mμ, log ϵ 4.22; ir., 5.58μ, 5.94μ, 6.11μ. Acetate: u.v., 277 mμ, log ϵ 4.24; ir., 5.58μ, 5.93μ, 6.11μ. Chloride: u.v., 277 mμ, log ϵ 4.18; ir., 5.61μ, 5.94μ, 6.13μ), and conversion of each in high yield to patulin phenylhydrazone by aqueous phenylhydrazine indicate the presence of a non-enolic hydroxyl group and exclude occurrence of enolization or isomerization during their preparation.

Patulin shows reactions (negative Schiff, positive Tollens, positive Fehling)[1] characteristic of a lactal ring. Lactal ring opening by phenylhydrazone formation usually unmasks a hydroxyl group and the conversion of patulin phenylhydrazone (IV, R = H) by treatment with sodium



(IV) (V) (VI)

acetate–acetic anhydride to patulin phenylhydrazone acetate (IV, R = Ac), m. p. 143° (calcd. for $C_{15}H_{14}O_4N_2$: C, 63.00; H, 4.93. Found: C, 63.40; H, 5.27) fits this interpretation. Infrared spectra of patulin phenylhydrazone (5.86μ, 6.04μ, 6.23μ) and its acetate (5.84μ, 6.00μ, 6.22μ) indicate retention of the doubly-unsaturated lactone system in these derivatives. Demonstration of a lactone ring in the phenylhydrazone and its acetate is shown by consumption of 1.05 and 1.92 equivalents, respectively, of sodium hydroxide. Dihydropatulin (V, X = OH) phenylhydrazone[4] contains only a singly-unsaturated lactone system (u.v., 380 mμ, log ϵ 4.55; 1.07 equivalents sodium hydroxide).

Treatment of patulin with warm excess thionyl chloride followed by sublimation furnishes unstable patulyl chloride (III, X = Cl) in 78% yield, m. p. 92–94° (calcd. for $C_7H_5O_3Cl$: C, 48.70; H, 2.92; Cl, 20.55. Found: C, 48.94; H, 2.63; Cl, 20.43); structural evidence given above. Patulyl chloride in anhydrous dioxane with palladium–barium sulfate catalyst absorbs 2.0 of moles hydrogen in two hours to give a neutral fraction which furnishes on distillation oily dihydrodesoxypatulin (V, X = H) in 34% yield, b. p. 90–95° (0.5 mm.). (Calcd. for $C_7H_8O_3$: sapon. equiv. 140.1. Found: 141.2); immediate Legal test; u.v., at 212 mμ, log ϵ 3.93; ir., 5.57μ, 6.01μ. Accordingly, dihydrodesoxypatulin contains a β,γ-unsaturated-γ-lactone system and its exact structure is established by hydrolysis in aqueous alcoholic sodium hydroxide to dihydrodesoxypatulinic acid (VI), identified by its well-known derivatives[2,3,4,5]: 2,4-dinitrophenylhydrazone, m. p. 193–195°; methyl ester 2,4-dinitrophenylhydrazone, m. p. and m. m. p. 149–150° (calcd. for $C_{14}H_{16}O_7N_4$: C, 47.70; H, 4.58. Found: C, 47.50; H, 5.02); *p*-phenylphenacyl ester, m. p. 124–127°.

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(2) Engel, Brzecki and Plattner, *Helv. Chim. Acta*, **32**, 1166, 1752 (1949).

(3) Woodward and Singh, THIS JOURNAL, **71**, 758 (1949).

(4) Bergel, Morrison, Moss and Rinderknecht, *J. Chem. Soc.*, 415 (1944).

(5) Acknowledgments are made gratefully to Professor Raistrick and the Therapeutic Research Corporation of Great Britain for the supply of patulin, to Professor Woodward and Dr. Singh for helpful discussions, spectral determinations on the phenylhydrazone derivatives and an authentic sample of the methyl ester dinitrophenylhydrazone, and to E. I. du Pont de Nemours and Co. for the Fellowship granted to one of us (F. L. W.).