and para-positions and it appears that V is not a good model for such compounds despite the fact that II seems to be a reasonable analog of nitrobenzene. The disparity can be resolved in the following way. The secular equation for II (and the corresponding activated complex I with X = $\overset{\oplus}{C}H_2$–) contains terms expressing the "resonance" integral β and the Coulombic integral q of the $\overset{\oplus}{C}H_2$–group. For a graded series of compounds ranging between II and benzene with various degrees of electronically deficient groups in place of $\overset{\oplus}{C}H_2$–, the q and β terms diminish to zero as one approaches benzene, but nonetheless, at all times, meta-substitution is predicted to be preferred. As a result, II is a good qualitative model for nitrobenzene or any similar substance where directing substituent exerts its influence by virtue of an electronically-deficient atom attached directly to the benzene ring.

On the other hand, in the transition between V and IV as β and q diminish, a changeover between prediction of meta- to ortho–para orientation must occur. Since the electronically-deficient atoms of the usual ortho-para substituting derivatives of VI (X = $-NO_2$, $-CO_2C_2H_5$, etc.) would be expected to have smaller q and β values than $\overset{\oplus}{C}H_2$–, it is possible that such groups on detailed analysis would be calculated to give different orientations from V. It will be interesting to determine whether electrophilic substitution of V or a suitable analog would actually occur in the meta-position as predicted.

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Effects of Hydrogenation upon the Microbiological Activities of N^{10}-Methylpteroylglutamic Acid, Aminopterin and A-Methopterin

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It has been found that hydrogenated folic acid has a growth-promoting activity for *Leuconostoc citrovorum 8081* which is intermediate between that of folic acid and that of leucovorin[1-3] and also intermediate between that of N^{10}-formylfolic acid and that of leucovorin.[3] Also, a commercial sample of aminopterin (4-aminopteroylglutamic acid), which inhibits the growth of *Leuconostoc citrovorum 8081*, was converted to a growth factor for this organism by hydrogenation under pressure.[4] It was later found that this growth-promoting activity was due to an impurity, but nevertheless the inhibitory action of pure aminopterin was decreased by hydrogenation.[5] These facts indicate that the state of oxidation of these compounds has important influence upon the microbiological activity.

Since hydrogenation at elevated temperature under pressure could conceivably cause degradation of the aminopterin molecule, it seemed worthwhile to carry out the hydrogenation under milder conditions and to extend the study to include N^{10}-methylpteroylglutamic acid and A-methopterin (N^{10}-methyl-4-aminopteroylglutamic acid). Accordingly, the compound (100 mg.) to be hydrogenated was suspended, without further purification, in water (25 ml.), and the *p*H was adjusted to 7.0–8.0 by adding a solution of sodium hydroxide, whereupon a homogeneous solution was obtained. Platinum oxide (50 mg.) was added, and hydrogenation was carried out at room temperature and atmospheric pressure. After approximately two moles of hydrogen had been taken up and hydrogenation appeared to be complete, the catalyst was removed by filtration through a bed of fullers earth on a sintered glass plate. The entire filtrate was subjected to freeze-drying, and this yielded a light-colored, fluffy, solid product which was stored under nitrogen. When compared with the spectra for pteroylglutamic acid and tetrahydropteroylglutamic acid,[6] spectra for the initial materials and the products of hydrogenation indicated that hydrogenation had been accomplished.

To determine the inhibitory activities of the initial materials and of the products of hydrogenation for *Streptococcus faecalis* R. the method of Mitchell and Snell[7] was used with slight modification, and folic acid was used as the growth factor. Constant levels of folic acid were used, and the quantity of test compound was varied. The final volume of medium per tube was 10 ml. Seventeen hours following inoculation the turbidity was determined by means of a Lumitron colorimeter with a 660 mμ filter. The resulting data are given in Table I. In all instances hydrogenation caused a considerable decrease in inhibitory activity.

TABLE I

EFFECT OF HYDROGENATION UPON THE INHIBITORY PROPERTIES OF CERTAIN FOLIC ACID ANTAGONISTS WITH *Streptococcus faecalis* R.

Compound[a]	Inhibition index[b] Folic acid (μg./10 ml.) 0.002	0.02	0.2	2.0
N^{10}-Methyl PGA	25	2.5	0.44	0.28
Hydrogenated N^{10}-methyl PGA	Less than 50% inhibition at all levels			
Aminopterin	26	2.8	1.5	0.34
Hydrogenated aminopterin	Less than 50% inhibition at all levels			
A-methopterin	25.5	2.5	0.25	0.04
Hydrogenated A-methopterin	45	70	26	11

[a] All compounds were used as the sodium salts. [b] Inhibition index = weight of agent required for half maximal inhibition/weight of folic acid.

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(2) H. P. Broquist, M. J. Fahrenbach, J. A. Brockman, Jr., E. L. R. Stokstad and T. H. Jukes, *ibid.*, **73**, 3535 (1951).

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(6) A. Pohland, E. H. Flynn, R. G. Jones and W. Shive, THIS JOURNAL, **73**, 3247 (1951).

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TABLE II

GROWTH-PROMOTING ACTIVITIES OF HYDROGENATED COMPOUNDS

		Transmission, %		
	μg./10 ml.	Hydrogenated N^{10}-methyl PGA	Hydrogenated aminopterin	Hydrogenated A-methopterin
For *S. faecalis* R.	0.1	100	53	100
	1.0	100	36	100
	10.0	68	25	100
	100.0	66	26	100
For *L. citrovorum 8081*	0.1	90	91	91
	1.0	92	90	93
	10.0	90	77	94
	100.0	78	33	90

The hydrogenated materials were also tested as growth factors for *Streptococcus faecalis* R. and *Leuconostoc citrovorum 8081*. For the former organism, the same basal medium was used as was used for the inhibition studies but no folic acid was added. For the latter organism, the basal medium and technique of Sauberlich[8] were used with the exceptions that no supplementary glycine and alanine were used and a Lumitron colorimeter with a 660 mμ filter was employed. Turbidity was determined after 17 hours. Data are given in Table II. It is quite likely that the growth-promoting activity is due to an impurity in the original compound as suggested by Weygand.

Acknowledgment.—The authors wish to thank Lederle Laboratories for supplying the folic acid, N^{10}-methylpteroylglutamic acid, aminopterin and A-methopterin used in these experiments.

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COMMUNICATIONS TO THE EDITOR

A NEW METHOD FOR THE PREPARATION OF THIO ACIDS AND APPLICATION TO PEPTIDE CHEMISTRY

Sir:

Although Pawlewski[1] demonstrated that thio acids were very active acylating agents, the methods of preparation which have been available heretofore[2] have not been suitable for making the acylaminothio acids which could be useful in peptide synthesis. By passing hydrogen sulfide into a solution of the mixed anhydrides,[3,4,5] RCOO-$COOC_2H_6$, in methylene chloride with an equivalent of triethylamine at −20° and warming to room temperature, we have obtained the thio acids, RCOSH.

In this manner we have prepared, in addition to thioacetic and thiobenzoic acids, *p*-phenylthiobenzoic acid, 88% yield (from the carboxylic acid), m.p. 90–92° (*Anal.* Calcd. for $C_{13}H_{10}OS$: C, 72.89; H, 4.71; S, 14.94. Found: C, 72.86; H, 4.83; S, 15.09); thiohippuric acid, 70% yield, m.p. 98–100° (*Anal.* Calcd. for $C_9H_9NO_2S$: C, 55.39; H, 4.65; N, 7.18; S, 16.40. Found: C, 55.30; H, 4.69; N, 6.79; S, 15.99); phthaloylthioglycine, 45% yield, m.p. 114–116° (*Anal.* Calcd. for $C_{10}H_7NO_3S$: C, 54.30; H, 3.19; S, 14.47. Found: C, 54.52; H, 3.32; S, 14.21).

When thiohippuric acid was warmed to 90–110° in dimethylformamide with *d,l*-alanine in a nitrogen atmosphere, hydrogen sulfide was rapidly evolved and there was obtained a 70% yield of hippuryl-alanine, m.p. 200–201.5°[6] and giving the correct elemental analysis.

Upon treatment of thiohippuric acid with Raney nickel which had been deactivated over acetone[7] there was obtained in one experiment, a 30% yield of hippuraldehyde,[8] isolated as the 2,4-dinitrophenylhydrazone, m.p. 200–202° (*Anal.* Calcd. for $C_{15}H_{13}N_5O_5$: C, 52.48; H, 3.82; N, 20.40. Found: C, 52.63; H, 3.78; N, 20.18).

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THE SYNTHESIS AND REACTIONS OF N-ACYL THIOL AMINO ACIDS

Sir:

Recent evidence that enzymatic acylations involve thiolacid derivatives as activated intermediates[1] has stimulated interest in similar thiol analogs of amino acids as possible participants in the physiological synthesis of peptides. By two

(1) For example, acetyl coenzyme A is considered to be a key intermediate in biological acetylations; F. Lynen, E. Reichert and L. Rueff, *Ann.*, **574**, 1 (1951); T. C. Chou and F. Lipmann, *J. Biol. Chem.*, **196**, 89 (1952).