PROCEEDINGS
OF THE
NATIONAL ACADEMY OF SCIENCES
Volume 27 September 15, 1941 Number 9

THE PYRIDINE ANALOG OF THIAMIN AND THE GROWTH OF FUNGI

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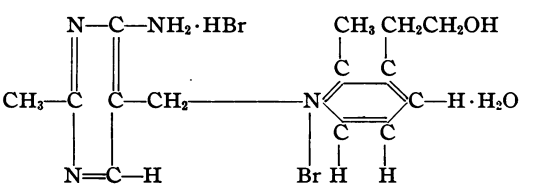
Communicated July 19, 1941

The concept of isosterism as applied to thiamin has been the subject of recent discussion. Schmelkes and Joiner[1] and Schmelkes[2] described the synthesis of a compound isosteric with thiamin and reported that it had some antineuretic activity. Baumgarten and Dornow[3] synthesized the same substance and reported its antineuretic activity to be about one twenty-sixth that of thiamin.

It is probable, however, that the compound described by Schmelkes and by Baumgarten and Dornow was an α-hydroxyethyl derivative instead of the assumed β-hydroxyethyl pyridine.[4] Tracy and Elderfield[5] synthesized the β-hydroxyethyl compound. Their compound is 1-(4-amino-2-methyl)-5-pyrimidylmethyl-2-methyl-3-(β-hydroxyethyl)-pyridinium bromide hydrobromide (monohydrate). The pyrimidine[6] portion of this compound is identical with that of thiamin; the pyridine portion differs from the thiazole part of thiamin as may be noted from the following formula



I was furnished this compound through the courtesy of Dr. Elderfield and N. J. Leonard. According to Leonard it is quite stable and does not undergo any interaction or decomposition when heated in water for any reasonable time.

I have tested this compound with three fungi which show thiamin deficiencies of various types. *Phycomyces Blakesleeanus* is unable to synthesize either the pyrimidine or thiazole portion of the thiamin molecule but if supplied with the two intermediates can combine them into the essential

thiamin molecule. *Pythiomorpha gonapodioides* is unable to synthesize the pyrimidine portion of the thiamin molecule but if furnished with the pyrimidine intermediate of thiamin is able to combine it with thiazole synthesized by the organism itself from the minerals, sugar and asparagine in the medium. *Phytophthora cinnamomi* is unable to synthesize either portion of the thiamin molecule and cannot combine the two intermediates even if they are supplied to it. *Phycomyces Blakesleeanus* grows if furnished with thiamin or the two intermediates; *Pythiomorpha gonapodioides* if supplied with thiamin or the pyrimidine portion of the thiamin molecule; *Phytophthora cinnamomi* must be supplied with molecular thiamin.

TABLE 1

DRY WEIGHT OF MYCELIUM PRODUCED IN SOLUTION OF MINERALS, ASPARAGINE AND DEXTROSE PLUS SUPPLEMENTS AS INDICATED

Phycomyces was grown 8 days at 20°C., *Pythiomorpha* and *Phytophthora* 11 days at 25°C.

ADDITIONS PER FLASK CONTAINING 25 ML. OF THE BASAL SOLUTION	AVERAGE DRY WT. PER FLASK MG. PHYCOMYCES	PHYTOPHTHORA	PYTHIOMORPHA
None	0.3	10.1	4.7
0.5 mμ mole of analog	0.2	10.2	42.7
1.0 mμ mole of analog	Almost no growth	6.7	68.4
2.0 mμ moles of analog	Almost no growth	7.9	0.7
10.0 mμ moles of analog	No growth	1.9	No growth
100.0 mμ moles of analog	No growth	1.4	No growth
1000.0 mμ moles of analog	No growth	1.2	No growth
2.0 mμ moles of analog + 100.0 mμ moles pyrimidine	Almost no growth	12.1	85.3
2.0 mμ moles of analog + 100.0 mμ moles of thiazole	139.5	6.5	39.9
0.5 mμ mole of thiamin	71.8	104.2	49.6
1.0 mμ mole of thiamin	124.5	133.3	74.6
2.0 mμ mole of thiamin	139.0	187.1	99.2

Each organism was grown in 25-ml. quantities of a solution containing per liter 50 g. glucose, 1.5 g. KH_2PO_4, 0.5 g. $MgSO_4.7H_2O$, 2 g. asparagine and the following mineral supplements in p. p. m. 0.005 B, 0.02 Cu, 0.1 Fe, 0.01 Ga, 0.01 Mn, 0.01 Mo, 0.09 Zn. To this solution various amounts of the pyridine analog, of thiamin, thiazole or pyrimidine were added as shown in tables 1 and 2. Solutions were sterilized for twenty minutes at twelve pounds pressure, and each treatment was carried out in triplicate. At the end of the period of growth the mycelium was removed from each flask, washed with distilled water, dried at 100°C. and weighed. The quantities of the various organic supplements added per flask are expressed in millimicromoles (mμ moles).

The growth of *Phycomyces* was not improved by the addition of the

pyridine analog to the basal solution. In fact, the analog was somewhat injurious. The pyridine analog was not effective when presented to the fungus in the presence of the pyrimidine intermediate of thiamin but good growth was obtained with a mixture of 2 mμ moles of the analog and 2 mμ moles of thiazole. The addition of 1.0 or 2.0 mμ moles of the analog was somewhat injurious and 10 mμ moles or more inhibited growth entirely. However, failure of the organism to use the analog was not the result of toxicity because excellent growth was obtained in the mixture of 2 mμ moles of the analog and 100 mμ moles of thiazole.

TABLE 2

DRY WEIGHT OF MYCELIUM OF *Phytophthora* AND *Pythiomorpha* PRODUCED IN A BASAL SOLUTION PLUS SUPPLEMENTS AS INDICATED. GROWN 10 DAYS AT 26°C.

ADDITIONS PER FLASK CONTAINING 25 ML. OF THE BASAL SOLUTION	AVERAGE DRY WT. PER FLASK MG. PHYTOPHTHORA	PYTHIOMORPHA
None	6.9	2.3
0.5 mμ mole of analog	1.9	35.3
1.0 mμ mole of analog	3.0	55.0
2.0 mμ moles of analog	2.4	0.3
10.0 mμ moles of analog	1.9	No growth
0.5 mμ mole of analog + 100.0 mμ moles pyrimidine	9.6	91.4
0.5 mμ mole of analog + 100.0 mμ moles thiazole	8.2	35.7
1.0 mμ mole of analog + 100.0 mμ moles pyrimidine	5.4	79.4
1.0 mμ mole of analog + 100.0 mμ moles thiazole	11.6	56.3
1.0 mμ mole of analog + 1.0 mμ mole thiamin	169.8	107.0
0.5 mμ mole thiamin	109.9	39.3
1.0 mμ mole thiamin	174.0	71.8
2.0 mμ moles thiamin	177.5	107.3

The ability of *Phycomyces* to utilize the appropriate portion of a thiamin analog has been observed previously. Robbins and Kavanagh[7] used a thiamin analog in which the thiazole portion of the molecule was identical with that of thiamin while the pyrimidine portion was not. Although ineffective alone, this compound was effective in mixture with pyrimidine. Schopfer[8] obtained similar results with two compounds which contain the vitamin thiazole. Bonner and Erickson[9] state that appropriate inactive vitamin analogs can serve as a source of vitamin pyrimidine. They found, however, that 2-chlora-isoaneurin-iodide did not serve as a source of thiazole in the presence of pyrimidine.

The growth of *Phytophthora* (table 1) was not improved by the addition of the pyridine analog to the basal medium. Mixtures of the analog and

thiazole or pyrimidine were ineffective. Distinct evidences of toxicity were observed in those flasks containing 10 mμ moles or more of the analog.

Pythiomorpha grew quite satisfactorily in those cultures supplemented with 0.5 or 1.0 mμ of the analog though it developed more slowly than in cultures with molecularly equivalent amounts of thiamin. Decided toxicity was observed in the cultures containing 2 mμ moles or more of the analog; in fact, no growth developed where 10 mμ moles or more were present. It is of interest to note that the addition of pyrimidine or thiazole materially reduced the toxicity of the analog. Evidently *Pythiomorpha* is able to utilize the pyrimidine portion of the analog molecule.

A second series of cultures (table 2) was carried out with *Pythiomorpha* and *Phytophthora* in which inoculum was obtained from a culture grown without growth-substances in the first experiment. This inoculum contained less thiamin and its intermediates than that used for the first series which was obtained from cultures growing on an agar medium containing thiamin. The results of the second series confirmed those of the first (table 2).

It appears that the pyridine analog is not effective as a substitute for thiamin for either *Phycomyces* or *Phytophthora*. No evidence of any benefit from the analog was observed though its toxicity prevents its use in large amounts where effectiveness of the order of one-hundredth or one-thousandth that of thiamin might be observed. Its effectiveness with *Pythiomorpha* is because the analog contains the normal pyrimidine intermediate of thiamin.

Both *Phycomyces* and *Pythiomorpha* are able to obtain pyrimidine from the analog. *Phycomyces* is apparently able to split off the pyrimidine from the analog molecule and combine it with thiazole thus obtaining the necessary thiamin. *Pythiomorpha* also seems able to split the analog molecule and secure pyrimidine which it combines into thiamin with thiazole synthesized from the sugar and minerals in the medium. The analog is of no use to *Phytophthora* which requires molecular thiamin and cannot synthesize any part of it or combine the intermediates even if they are furnished.

[1] Schmelkes, F. C., and Joiner, R. R., *J. Am. Chem. Soc.*, **61**, 2562 (1939).

[2] Schmelkes, F. C., *Science*, **90**, 113 (1939).

[3] Baumgarten, P., and Dornow, A., *Ber. deutsch. chem. Gesell.*, **73**, 44 (1940).

[4] Baumgarten, P., and Dornow, A., *Ibid.*, **73**, 353 (1940).

[5] Tracy, A. H., and Elderfield, R. C., *Science*, **92**, 180 (1940).

[6] The terms pyrimidine and thiazole are used in this paper to refer to the two intermediates of thiamin.

[7] Robbins, W. J., and Kavanagh, F., *Proc. Nat. Acad. Sci.*, **24**, 141 (1938).

[8] Schopfer, W. H., *Bull. Soc. bot. Suisse*, **47**, 460 (1937).

[9] Bonner, J., and Erickson, J., *Am. Jour. Bot.*, **25** (1938).