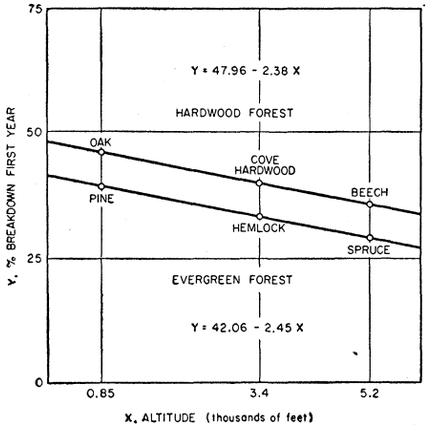
Effect of altitude is linear (2.4 percent difference in rate of breakdown per 1000 ft elevation or approximately 1 percent per degree Fahrenheit) with nonsignificant difference in regression slope between deciduous and evergreen forests. This compares closely with Mikola's differences of 1.75 percent per degree centigrade in first-year breakdown of pine needles in pine and spruce forests of northern and southern Finland, but is slightly less than the difference of 2.4 percent per degree centigrade for birch leaves (*13*). The consistent difference in percentage breakdown between evergreen and deciduous forests is about 6 percent, a difference slightly greater than would be expected from the microclimatic temperature records (*14*). It remains to be seen to what degree the differences between these contrasting adjacent stands are due to their microclimate and to what degree due to differences in microbiology.

Species differences were highly significant (Fig. 2) and consistent (Fig. 1). Most beech leaves remained unfragmented for the full year, with their degree of discoloration and perforation correlated with their weight losses (ranging from 12 percent under spruce to 29 percent under oak). Mulberry not only lost far more weight (57 to 68 percent), but the material remaining in the bags was rapidly darkening and losing form in only 4 mo. Aggregates of leaves had been changed by the end of the year to black masses of humus, which would have moved into the *H* or A_1 layer of the soil if not confined in the bags. White oak, Shumard red oak, and sugar maple were intermediate between mulberry and beech in weight loss, discoloration, and fragmentation. Similar several-fold species differences in rates of breakdown have been shown in laboratory experiments and have been related to organic and inorganic chemistry of the leaves (*15*).



ANALYSIS OF VARIANCE

SOURCE	d.f.	M.S.
COVER	1	282.1**
ALTITUDE	2	
LINEAR	1	556.0**
RESIDUAL	1	5.8
ERROR I	2	0.65
SPECIES	4	1471.7**
SP. x COVER	4	5.55
SP. x ALT.	8	5.57
ERROR II	8	20.54

**HIGHLY SIGNIFICANT WITH RESPECT TO EITHER ERROR I OR ERROR II.

Fig. 2. Regression of percentage breakdown, averaged for five species of leaves, on altitude of three evergreen and three deciduous forest stands in which leaves were exposed for 1 yr; split plot analysis of variance showing highly significant effects of evergreen versus deciduous cover, altitude, and species. Deviations of plot means (circles) from fitted regression slopes are not significant. Error II may include high order interactions, but these are small compared with main effects.

The surprisingly small difference between oak and sugar maple in litter bags, compared with more rapid disappearance of maple in certain natural forests, might be due to the absence or exclusion of large earthworms (*10*) which apparently select sugar maple when present in mixture with oak (*11*). Because of confinement of the leaves and their fragments, and restricted access of the larger forest floor fauna, the present results are not absolute measurements of breakdown of forest litter under natural conditions, but provide for estimates of relative rates under standardized conditions.

It remains to be seen whether losses in later years will show approximately constant percentage breakdown so that they fit theoretical models assuming linear differential equations with constant coefficients (*4*, *5*, *7*), or whether quantitative models for the development of forest floor material, the cycling of nutrients, and the dispersal of radioactive contamination will have to employ differential equations with variable coefficients (*16*).

R. E. SHANKS

Botany Department,
University of Tennessee, Knoxville

J. S. OLSON

Health Physics Division,
*Oak Ridge National Laboratory,**
Oak Ridge, Tennessee

References and Notes

1. S. A. Cain, *Bull. Torrey Botan. Club* **70**, 213 (1943); E. L. Braun, *Deciduous Forests of Eastern North America* (Blakiston, New York, 1950); R. H. Whittaker, *Ecol. Monographs* **26**, 1 (1956).
2. H. R. DeSelm and R. E. Shanks, *Proc. IX Intern. Botan. Congr.*, Montreal, in press.
3. J. S. Olson, *Proc. IX Intern. Botan. Congr.* **2**, 287 (1959).
4. ———, *Oak Ridge Natl. Lab. Rept No. ORNL 2806, 41–45* (1959).
5. J. S. Olson, W. C. Cate, D. A. Crossley, Jr., M. P. Hoglund, R. B. Neel, H. D. Waller, M. Witkamp, J. P. Witherspoon, J. A. Wolfe, *Oak Ridge Natl. Lab. Rept. No. ORNL 2994, 167–185* (1960).
6. J. T. McGinnis, thesis, University of Tennessee (1958).
7. H. Jenny, S. P. Gessel, F. T. Bingham, *Soil Sci.* **68**, 419 (1949).
8. R. E. Shanks, *Ecology* **35**, 354 (1954).
9. J. G. Falconer, J. W. Wright, H. W. Beall, *Am. J. Botany* **20**, 196 (1933); H. A. Lunt, *J. Forestry* **33**, 607 (1935); J. W. Johnston, Jr., thesis, Harvard University (1935); M. Ohmasa and K. Mori, *Bull. For. Exptl. Sta. Tokyo-Fu* **3**, 39 (1937); A. Nömmik, *Bodenk. u. Pflanzenernähr.* **8**, 77 (1938); W. Wittich, *Forstarchiv* **15**, 96 (1939); ——— *ibid.* **19**, 1 (1943); F. G. Gustafson, *Plant Physiol.* **18**, 704 (1943); B. B. Coldwell and W. A. DeLong, *Sci. Agr.* **30**, 456 (1950); P. Mikola, *Communs. Inst. Forest. Fenn.* **43**, 1 (1954); C. Kucera, *Ecology* **40**, 485 (1959); M. Witkamp, *Mededel. Inst. Toegepast Biol. Onderzoek. Nat.* **46**, 1 (1960); ——— and J. Van der Drift, *Plant and Soil*, in press; P. W. Murphy, in preparation.
10. K. L. Bocock and O. J. W. Gilbert, *Plant and Soil* **9**, 179 (1957); K. L. Bocock, O. J. W. Gilbert, C. K. Capstick, D. C. Twinn, J. S. Waid, M. J. Woodman, *J. Soil Sci.* **11**, 1 (1960).
11. C. E. Olmsted, unpublished experiments at Lake Geneva, Wis.
12. D. A. Crossley, Jr., unpublished data, Oak Ridge National Laboratory.
13. P. Mikola, *Oikos* **11**, 161 (1960).
14. R. E. Shanks, *Ecology* **37**, 1 (1956).
15. E. Melin, *ibid.* **11**, 72 (1930); W. M. Broadfoot and W. H. Pierre, *Soil Sci.* **48**, 329 (1939).
16. This work is contribution N. Ser. 221 from the Botanical Laboratory, University of Tennessee, and contribution 38 from the Ecology Section of the Oak Ridge National Laboratory; it was supported by contract No. AEC AT-(40-1)-2077 with the Environmental Sciences Branch, Division of Biology and Medicine, U.S. Atomic Energy Commission, and by contract No. W-7405-eng-26 with the Commission.
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A New Thiamine Derivative, S-Benzoylthiamine O-Monophosphate

Abstract. S-Benzoylthiamine O-monophosphate has been synthesized, and its physicochemical and biological properties have been investigated. It is a stable crystalline substance, it exerts thiamine activity approximately equivalent to that of thiamine hydrochloride in thiamine-requiring microorganisms, and it is easily absorbed in organisms, particularly by oral administration.

Recently, Fujiwara and others (*1*) reported on thiamine alkyldisulfides, including thiamine propyldisulfide, which were absorbed in organisms more easily than thiamine hydrochloride. In the course of studies on phosphoric acid derivatives of thiamine, we found that a new derivative of thiamine monophosphate, S-benzoylthiamine O-monophosphate (BTMP), exhibited similar ease in absorbability in organisms by oral administration.

S-Benzoylthiamine O-monophosphate,

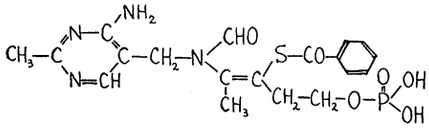

Fig. 1. Chemical structure of BTMP.

$C_{19}H_{23}O_6N_4SP$, molecular weight of 466.47, has the chemical structure shown in Fig. 1. It is a colorless, odorless, crystalline substance of prism form, which melts at about 195°C with decomposition. It is sparingly soluble in benzene, chloroform, dioxane, pyridine, ethanol, and methanol and readily soluble in glacial acetic acid. Its solubilities in water are 0.004 g/ml at *p*H 4.05 and more than 1 g/ml at *p*H 8.0, the former *p*H corresponding to its isoelectric point. Although it is an amphoteric substance, as are protein and amino acids, it is almost nonhygroscopic. When crystals were exposed directly to the sun in air for a period of 23 days to observe coloring, almost no coloring was produced as compared to the control.

The compound is very stable in aqueous solutions under acid conditions, as expected from the chemical structure. An aqueous solution in a concentration of 5 mg/ml was heated at 100°C at various *p*H's, with the result that more than 90 percent of the initial amount remained undecomposed at a *p*H between 1.0 and 5.6 after 2 hr.

It has a thiamine potency in microorganisms and animals at least as high as an equimolar amount of thiamine hydrochloride. It is more easily absorbed in the body than thiamine hydrochloride, and administration results in higher thiamine and cocarboxylase levels in organs; moreover, these levels last for a longer period of time. This characteristic is particularly remarkable when the compound is administered orally. It does not cause any unfavorable symptom in animals such as thiamine hydrochloride does (the LD_{50} of the former is larger than that of the latter), especially after intravenous and intraperitoneal injections.

The new derivative exerted thiamine activity approximately equivalent to thiamine hydrochloride in a microorganism, *Lactobacillus fermenti* 36, in birds, *Uroloncha striata var. domestica,* and in a pigeon.

As aneurinase I, a culture broth of *Bacillus thiaminolyticus* Matsukawa et Misawa (species MM) was used, and as aneurinase II that of *B. aneurinolyticus* Kimura et Aoyama (species KA). The remaining thiamine activity was measured after incubation at *p*H 7.5 at 37°C for 2 hr, with the result that 97.3 and 95.3 percent, respectively, remained with aneurinases I and II. The thiamine activities in the case of thiamine hydrochloride were 57.0 and 65.2 percent, respectively.

The LD_{50} by oral administration in mice, dd-strain hybrid, weighing 14 to 16 g was 15 g/kg of body weight (95 percent confidence limit, 13.3 to 16.9 g/kg) (compare 9 g/kg for thiamine hydrochloride), calculated according to the method of Litchfield and Wilcoxon (*2*). The LD_{50} by intravenous injection in male mice, dd-strain hybrid, weighing 14 to 16 g, was 2.2 g/kg of body weight (thiamine hydrochloride, 0.1 g/kg). The LD_{50} by intraperitoneal injection in female mice, dd-strain, weighing 16 to 18 g, was 1.81 g/kg of body weight. In cats anesthetized with pentobarbital, spinal injection of BTMP at doses from 0.1 to 0.3 mg/kg of body weight caused almost no change in respiration or blood pressure; even at larger doses (as much as 0.5 to 1.2 mg/kg of body weight) BTMP did not produce any remarkable reaction, although in a few cases the rise or fall of blood pressure and the increase or expiration of respiration were noted in the same way as with thiamine hydrochloride.

Table 1. Total thiamine levels in blood in dogs, after oral administration of S-benzoylthiamine O-monophosphate and thiamine hydrochloride.

Dose (mg/kg of body weight)	Thiamine level (μg/100 ml)					
	Before administration	Hours after administration				
		1	2	4	6	10
		S-Benzoylthiamine O-monophosphate				
1	7.3	43.3	28.1	18.1	16.5	14.2
3	7.7	109.3	87.0	59.2	43.7	30.5
		Thiamine hydrochloride				
1	7.0	18.0	12.0	10.0	7.6	7.1
3	8.0	26.7	22.4	14.7	12.0	8.1

Table 2. Urinary thiamine excretion in human beings 24 hr after oral administration of S-benzoylthiamine O-monophosphate and thiamine hydrochloride at various doses (in milligrams).

5 mg	15 mg	25 mg	50 mg	100 mg
S-Benzoylthiamine O-monophosphate				
1.72	3.17	5.83	11.92	22.30
Thiamine hydrochloride				
1.45	1.68	1.94	2.17	2.90

The total thiamine levels in blood in dogs after oral administration of various amounts of BTMP and the urinary thiamine excretion in human beings after oral administration are illustrated in Tables 1 and 2 in comparison with results for thiamine hydrochloride. The thiamine assay was conducted according to the method of Fujiwara and Matsui (*3*). The doses in the tables are expressed by the amount of thiamine hydrochloride equimolar to the given amount of BTMP. Blood cocarboxylase levels determined according to the method of Kay *et al.* (*4*) were higher after administration of BTMP through various routes than after administration of hydrochloride in equimolar amounts.

Investigations of the therapeutic applications of BTMP are in progress in various hospitals in Japan (*5*).

TADAO WADA, HIROMU TAKAGI, HARUHIKO MINAKAMI, WATARU HAMANAKA, KOICHI OKAMOTO, AKIRA ITO, YOSHIRO SAHASHI

Takamine Laboratory, Sankyo Company, Limited, Tokyo, Japan

References and Notes

1. M. Fujiwara and H. Watanabe, *Proc. Japan Acad.* **28**, 156 (1952); T. Matsukawa, *ibid.* **28**, 146 (1952).
2. J. T. Litchfield, Jr., and F. Wilcoxon, *J. Pharmacol. Exptl. Therap.* **96**, 99 (1949).
3. M. Fujiwara and K. Matsui, *Anal. Chem.* **25**, 810 (1953).
4. W. W. Kay *et al.*, *Biochem. J.* **62**, 601 (1956).
5. A detailed report on the results of our study is in preparation.

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