

selective hydrogenation while at an iodine value of approximately 48 these values increased to 21.6 and 37.7, respectively.

#### Acknowledgment

The authors wish to acknowledge the assistance of R. T. O'Connor, Elizabeth R. McCall, Elsie F. DuPré, and Dorothy C. Heinzelman, who obtained the infrared and ultraviolet absorption data used in calculating the composition of the oil samples.

#### REFERENCES

1. Allen, R. R., *J. Am. Oil Chemists' Soc.*, **33**, 301-304 (1956).
2. Allen, R. R., and Kiess, A. A., *J. Am. Oil Chemists' Soc.*, **33**, 355-359 (1956).
3. Allen, R. R., and Kiess, A. A., *J. Am. Oil Chemists' Soc.*, **32**, 400-405 (1955).

4. American Oil Chemists' Society, "Official and Tentative Methods," 2nd ed., rev. to 1956, Chicago, 1946-56.
5. Bailey, A. E., *J. Am. Oil Chemists' Soc.*, **26**, 644-648 (1949).
6. Bailey, A. E., "Industrial Oil and Fat Products," 2nd ed., p. 721-722, New York, Interscience Publishers Inc. (1951).
7. Bailey, A. E., Feuge, R. O., and Smith, B. A., *Oil & Soaps*, **19**, 169-176 (1942).
8. Boelhouwer, C., Gerckens, J., Lie, O. T., and Waterman, H. I., *J. Am. Oil Chemists' Soc.*, **30**, 59-61 (1953).
9. Corcoran, G. B., *Anal. Chem.*, **28**, 168-171 (1956).
10. Feuge, R. O., Cousins, E. R., Fore, S. P., DuPré, E. F., and O'Connor, R. T., *J. Am. Oil Chemists' Soc.*, **30**, 454-460 (1953).
11. Feuge, R. O., Pepper, M. B. Jr., O'Connor, R. T., and Field, E. T., *J. Am. Oil Chemists' Soc.*, **28**, 420-426 (1951).
12. Higuchi, T., Hill, N. C., and Corcoran, G. B., *Anal. Chem.*, **24**, 491-493 (1952).
13. Hilditch, T. P., and Vidyarthi, N. L., *Proc., Roy. Soc. London*, **A122**, 552-570 (1929).
14. Lewkowitsch, J., "Chemical Technology and Analysis of Oils, Fats, and Waxes," 5th ed., Vol. 1, p. 192, New York, Macmillan, 1913.
15. Moore, C. W., *J. Soc. Chem. Ind.*, **38**, 320-325T (1919).
16. Swern, Daniel, Knight, H. B., Shreve, O. D., and Heether, M. R., *J. Am. Oil Chemists' Soc.*, **27**, 17-21 (1950).

[Received November 6, 1957]

## Antioxidative Activity of Derivatives of Vitamin B<sub>6</sub> and Structurally Related Compounds<sup>1</sup>

TAKETAMI SAKURAGI and FRED A. KUMMEROW, Department of Food Technology, University of Illinois, Urbana, Illinois

VITAMIN B<sub>6</sub> is believed to serve as a physiological antioxidant (1, 2, 3) and has also been reported to serve as an antioxidant for vitamin A *in vitro* (4).

Since the 3-hydroxyl group of the vitamin molecule may be responsible for the reported antioxygenic activity, an attempt was made to prepare pyridoxine 5-monopalmitate, which is completely soluble in fats, contains a free 3-hydroxyl group (5). Similarly ethyl *N*-pyridoxyl-*p*-aminobenzoate<sup>2</sup> (6) and pyridoxal isonicotinoylhydrazone (7) are of interest as possible oxidation inhibitors for fats. The latter compound is also known to form a chelate complex with various metallic ions, such as copper (8), and this property might serve to eliminate pro-oxidative metallic ions from fats.

In the present study various *N*-hydroxybenzyl and *N*-hydroxybenzylidene compounds were synthesized as preparations structurally related to vitamin B<sub>6</sub> derivatives, and they were tested for antioxidative activity in lard at 37°C. and at 80°C.

#### Experimental

**Test Compounds.** The synthesis of pyridoxine 5-monopalmitate as well as ethyl *N*-pyridoxyl-*p*-aminobenzoate<sup>2</sup> has been reported previously (5, 6). The derivatives of ethyl *N*-hydroxybenzyl-*p*-aminobenzoate were prepared according to the following procedures, using ethyl *p*-aminobenzoate and the respectively substituted benzaldehyde *via* reductive *N*-alkylation. Three and three-tenths grams of ethyl *p*-aminobenzoate and an equimolar amount of the properly substituted benzaldehyde were dissolved in 60 ml. of a mixture of methanol and dioxane (1:1, v/v). The solution was then hydrogenated under 20 lbs. of hydrogen pressure in the presence of 0.5 g. of platinum oxide catalyst at room temperature for 1 hr. After hydrogenation the catalyst was removed by filtra-

tion, and water was added to the filtrate until it showed slight turbidity. Upon standing, the product crystallized.

Ethyl *N*-anisyl-*p*-aminobenzoate (m.p., 128.5-130.0°C.) was recrystallized from isopropanol-methanol (9).

Ethyl *N*-(*p*-hydroxybenzyl)-*p*-aminobenzoate was recrystallized from methanol-water. M.p., 142.0-142.5°C. *Anal.* Calcd. for C<sub>16</sub>H<sub>17</sub>NO<sub>3</sub>: C, 70.83; H, 6.32; N, 5.16. Found: C, 71.18; H, 6.41; N, 5.11.

Ethyl *N*-salicyl-*p*-aminobenzoate was recrystallized from methanol. M.p., 146.5-148.0°C. *Anal.* Calcd. for C<sub>16</sub>H<sub>17</sub>NO<sub>3</sub>: C, 70.83; H, 6.32; N, 5.16. Found: C, 71.04; H, 6.50; N, 5.24.

Ethyl *N*-vanillyl-*p*-aminobenzoate was recrystallized from isopropanol-methanol. M.P., 149.0-150.0°C. *Anal.* Calcd. for C<sub>17</sub>H<sub>19</sub>NO<sub>4</sub>: C, 67.75; H, 6.36; N, 4.65. Found: C, 67.85; H, 6.58; N, 4.85.

*N*-Vanillyl-β-phenylethylamine hydrochloride was also prepared in a similar manner from vanillin and 2-phenylethylamine. The hydrogenation product was treated with hydrogen chloride, and the hydrochloride was recrystallized from ethanol-ether. M.p., 185.5-186.5°C. *Anal.* Calcd. for C<sub>16</sub>H<sub>19</sub>NO<sub>2</sub>·HCl: C, 65.41; H, 6.86; N, 4.77. Found: C, 65.07; H, 7.08; N, 4.55.

The isonicotinoylhydrazones and the nicotinoylhydrazones of pyridoxal, salicylaldehyde, and vanillin were prepared by treating the hydrazide with the proper aldehyde in ethanol-water in the presence of sodium acetate as a catalyst (7). The products were recrystallized from ethanol-water. Pyridoxal isonicotinoylhydrazone melted at 263.0°C. (decomposition) (7). Salicylaldehyde isonicotinoylhydrazone melted at 249.0-251.0°C. (10). The isonicotinoylhydrazone and the nicotinoylhydrazone of vanillin melted at 230.0°C. and 213.0-215.0°C., respectively (11, 12).

The synthesis of 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde isonicotinoylhydrazone (BHB-INH) was carried out as follows. Twenty-five grams of 2,6-di-*tert*-butylphenol were treated with hexamethylene tetramine in glycerine containing boric acid, followed by diluted sulfuric acid as described by Duff (15). Five grams of 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde were ob-

<sup>1</sup> This work was supported by Research Grant No. A-257 from the National Institutes of Health, U. S. Public Health Service, Department of Health, Education, and Welfare.

<sup>2</sup> The term "pyridoxyl" denotes the 2-methyl-3-hydroxy-5-hydroxymethyl-4-pyridylmethyl radical. The term "pyridoxylidene" denotes the 2-methyl-3-hydroxy-5-hydroxymethyl-4-pyridylmethylene radical.

tained after recrystallization from 95% ethanol.<sup>3</sup> M.p., 188.0–189.5°C. *Anal.* Calcd. for C<sub>15</sub>H<sub>22</sub>O<sub>2</sub>: C, 76.88; H, 9.47. Found: C, 76.15; H, 9.44. Two and three-tenths grams of 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde and 1.4 g. of isonicotinoylhydrazide were mixed in 35 ml. of 95% ethanol and refluxed for 40 min. in the presence of about 100 mg. of sodium acetate as a catalyst. Toward the end of this period fine needle-like crystals began to separate. The mixture was then allowed to stand at room temperature for about 24 hrs., and the precipitate was collected. After washing with ether and water, recrystallization was effected from pyridine-water. Yield: 2.7 g. M.p., 300.0°C. *Anal.* Calcd. for C<sub>21</sub>H<sub>27</sub>N<sub>3</sub>O<sub>2</sub>: C, 71.35; H, 7.70. Found: C, 71.74; H, 7.82.

**Test for Antioxidative Activity.** The antioxidative activity of various *N*-alkyl-*p*-aminobenzoates, isonicotinoylhydrazones, and other preparations was compared at 37°C. and at 80°C., using "Stripped Lard"<sup>4</sup> as a substrate. "Stripped Lard" was prepared from prime steam lard from which most of the tocopherols had been removed by molecular distillation. The fresh "Stripped Lard" had a peroxide value of less than 1 and an iodine value of 62.0.

Twenty grams of the lard were placed in a shallow beaker (inside diameter: 59 ± 1 mm.), and the test compound was added as a solution in 3 ml. of ethanol, in most cases, at a level of 1 μmole per gram of the substrate. Three ml. of ethanol without antioxidant were added to 20 g. of lard to serve as a blank control. The hydrochlorides of ethyl *N*-pyridoxyl-*p*-aminobenzoate and *N*-vanillyl-β-phenylethylamine originally prepared were treated with sodium carbonate, and the free amines thus liberated were used for the test. After thorough mixing, the solvent was removed on a water bath. When no further odor of alcohol was apparent, the beakers were transferred to an incubator kept at 37°C. or 80°C.

The antioxidative potency was compared on the basis of a peroxide value, which was determined at proper intervals of time according to the procedure of Lundberg *et al.* (13) and expressed as millimoles of peroxide per kilogram of fat. Throughout this study, comparison was made within a set of groups which were run concurrently. To test the effect of metallic ions on antioxidant activity, cupric acetate or ferric chloride dissolved in ethanol was used as a source of metallic ions at a level of 10 p.p.m. as Cu<sup>++</sup> or Fe<sup>+++</sup>.

### Results

Ethyl *N*-anisyl- and *N*-salicyl-*p*-aminobenzoates showed no or little antioxygenic activity; ethyl *N*-(*p*-hydroxybenzyl)- and *N*-pyridoxyl-*p*-aminobenzoates<sup>2</sup> possessed some activity, and *N*-vanillyl-*p*-aminobenzoate was the strongest oxidation inhibitor among the *N*-alkylaminobenzoates tested (Figure 1). *N*-vanillyl-β-phenylethylamine was also found to be an antioxidant although the potency was weaker than the *p*-aminobenzoate (Figure 1).

Under identical conditions pyridoxine 5-monopalmitate (5) was completely lacking in antioxygenic

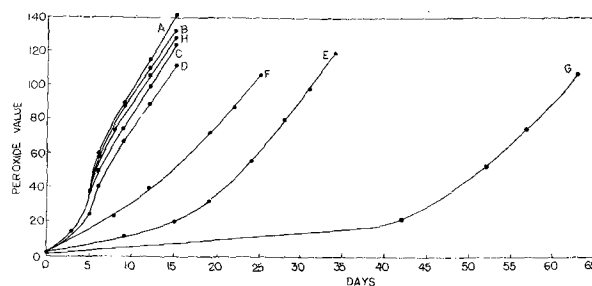


Fig. 1. Stability of lard containing (A) none, (B) ethyl *N*-anisyl-*p*-aminobenzoate, (C) ethyl *N*-salicyl-*p*-aminobenzoate, (D) ethyl *N*-pyridoxyl-*p*-aminobenzoate or ethyl *N*-(*p*-hydroxybenzyl)-*p*-aminobenzoate, (E) ethyl *N*-vanillyl-*p*-aminobenzoate, (F) *N*-vanillyl-β-phenylethylamine, (G) vanillin isonicotinoylhydrazone or vanillin nicotinoylhydrazone, and (H) ascorbyl palmitate. The test compound was added at a level of 1 μmole per gram of the substrate, and the test was conducted at 37°C. The peroxide value was expressed as millimoles of peroxide per kilogram of fat.

properties. It was also found that pyridoxine 5-monopalmitate as well as ethyl *N*-pyridoxyl-*p*-aminobenzoate at a level of 0.01 m mole per gram in a vitamin A preparation (Myvax<sup>4</sup>) failed to inhibit the oxidation of the vitamin when tested according to the procedure reported by Spruyt (14) at 37°C.

Various *N*-benzylidene and *N*-pyridoxylidene<sup>2</sup> compounds were tested in the presence of cupric and ferric ions in lard (Figure 2). In the presence of 10

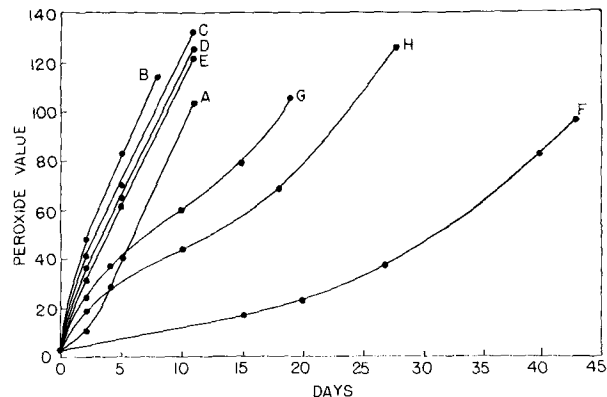


Fig. 2. Stability of lard containing (A) none, (B) Cu<sup>++</sup> or Fe<sup>+++</sup>, (C) Cu<sup>++</sup> plus isonicotinoylhydrazide, (D) Cu<sup>++</sup> plus salicylaldehyde isonicotinoylhydrazone, (E) Cu<sup>++</sup> plus pyridoxal isonicotinoylhydrazone or Cu<sup>++</sup> plus vanillin isonicotinoylhydrazone or Cu<sup>++</sup> plus vanillin nicotinoylhydrazone, (G) Fe<sup>+++</sup> plus vanillin nicotinoylhydrazone, and (H) Fe<sup>+++</sup> plus vanillin isonicotinoylhydrazone. The level of the metallic ion was 10 p.p.m.; the test compound was added at a level of 1 μmole per gram of the substrate, and the test was conducted at 37°C. The peroxide value was expressed as millimoles of peroxide per kilogram of fat.

p.p.m. of Cu<sup>++</sup>, salicylaldehyde isonicotinoylhydrazone, pyridoxal isonicotinoylhydrazone, and free isonicotinoylhydrazide failed to prevent oxidation. Strong protection from oxidation was however noted when vanillin isonicotinoylhydrazone or the nicotinoylhydrazone was used. These two compounds were also capable of preventing oxidation in the presence of Fe<sup>+++</sup>. In this case better protection resulted with isonicotinoylhydrazone than with nicotinoylhydrazone. Vanillin isonicotinoylhydrazone also served as

<sup>3</sup> This procedure was originally reported for *ortho*-formylation of phenols. Since no *ortho*-position was available in 2,6-di-*tert*-butylphenol, formylation took place at the *para*-position to give 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde. When an ethanolic solution of the reaction product was treated with ethanolic potassium hydroxide, no yellow color was developed, indicating that an *ortho*-hydroxybenzaldehyde was not present in the reaction product. This aldehyde has also been prepared from butylated hydroxytoluene through oxidation with bromine (27).

<sup>4</sup> Kindly supplied by Distillation Products Industries, Rochester, N. Y.

an antioxidant in the absence of metallic ions (Figure 1). Free vanillin as well as free ethyl *p*-aminobenzoate were inactive in the presence or absence of metallic ions.

The activity of butylated hydroxytoluene (BHT) and 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde isonicotinoylhydrazone (BHB-INH) was compared at 80°C. (Figure 3). In the absence of Cu<sup>++</sup>, BHB-INH prevented oxidation of lard more efficiently than BHT on a molar basis, indicating that an isonicotinoylhydrazonomethyl group potentiated the phenol better than a methyl group. Because of accelerated decomposition of peroxide by the metallic ion, lard showed a maximum peroxide value (millimoles of peroxide per kilogram of fat) of only 40–45 in the presence of 10 p.p.m. of Cu<sup>++</sup> at 80°C. Even in the presence of BHT at a level of 1 μmole per gram of substrate, when 10 p.p.m. of Cu<sup>++</sup> was present, lard became strongly rancid within 10 hrs. of incubation, and the oxidation pattern was essentially identical with that of the sample containing the metal but no antioxidant. On the other hand, BHB-INH significantly depressed oxidation of lard containing copper at the initial stage, possibly through removal of the metallic ion by chelation; and, as observed in the control group, while oxidation proceeded, the peroxide value increased beyond the level of 40–45 (Figure 3). The results thus indicated that, under the experimental conditions, the effect of BHT was totally masked by the pro-oxidative nature of copper whereas BHB-INH eliminated the metallic ion from the oxidation system and behaved as an antioxidant.

The activity of BHT and BHB-INH was also tested at a level of 1/6 μmole per gram of lard in the presence of 10 p.p.m. of Cu<sup>++</sup> at 37°C. The sample containing BHT took 135 hrs. to reach a peroxide value of 50, and the sample containing BHB-INH required 180 hrs. to reach the same peroxide level.

### Discussion

In the present study pyridoxine 5-monopalmitate (5) was found to be totally lacking in antioxidant activity in lard as well as in a vitamin A preparation at 37°C. although pyridoxine has been shown to possess antioxidative activity in fish oil (4). During the course of studies on various *N*-pyridoxylamines<sup>2</sup> (6) it was noted that ethyl *N*-pyridoxylaminobenzoates were readily oxidized to the corresponding *N*-pyridoxylideneaminobenzoates,<sup>2</sup> which are deeply yellow compounds. This reaction is analogous to the one which takes place at the similar linkage in the molecule of folic acid (16, 17) and in *N*-benzyl-*p*-aminobenzoic acid (16) upon oxidation. When ethyl *N*-pyridoxyl-*p*-aminobenzoate was dissolved in autoxidized ethyl linoleate which had a peroxide value of approximately 600 and incubated at 37°C. for a few days, a distinctive yellow color developed. This suggested that ethyl *N*-pyridoxyl-*p*-aminobenzoate and structurally related compounds could form the corresponding aldimines under these conditions. If ready disposal of hydrogen atoms is involved in the oxidation-inhibiting mechanism of an antioxidant, the *N*-pyridoxyl-*p*-aminobenzoate might also serve as an antioxidant. The experimental results however failed to support this assumption (Figure 1).

Ethyl *N*-vanillyl-*p*-aminobenzoate was a relatively strong antioxidant despite the fact that ethyl *N*-pyridoxyl-*p*-aminobenzoate showed little antioxidative ac-

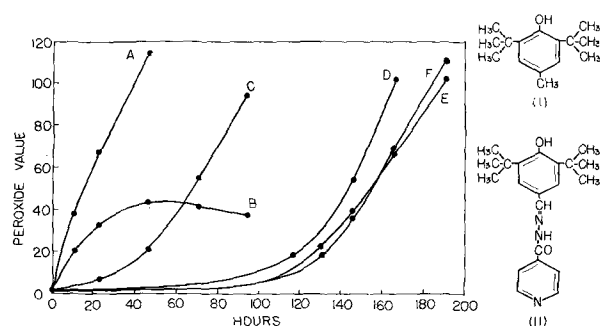


FIG. 3. Stability of lard containing (A) none, (B) Cu<sup>++</sup> plus butylated hydroxytoluene (BHT) (I), (C) Cu<sup>++</sup> plus 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde isonicotinoylhydrazone (BHB-INH) (II), (D) BHT, (E) BHB-INH, and (F) an equimolar mixture of BHT and BHB-INH. The oxidation pattern of the lard containing no antioxidant but Cu<sup>++</sup> was essentially identical with (B). The level of the metallic ion was 10 p.p.m.; the test compound was added at a level of 1 μmole per gram of the substrate, and the test was conducted at 80°C. The peroxide value was expressed as millimoles of peroxide per kilogram of fat.

tivity. The substituted groups between these two compounds are similar. They have a hydroxyl group at an *ortho* or *para* position to the *N*-(*p*-carbethoxyphenyl)-aminomethyl group, and they contain a methyl or methoxyl group at an *ortho* position to the hydroxyl. It is known that the antioxygenic property at a phenolic hydroxyl group is imparted by a methyl group more efficiently than a methoxyl group located at an *ortho* position to the hydroxyl (18). As observed with ethyl *N*-salicyl-*p*-aminobenzoate and the *N*-(*p*-hydroxybenzyl)-*p*-aminobenzoate (Figure 1), it appears conceivable that a compound possessing a hydroxyl group at the *para* position to the substituted aminomethyl group may show somewhat stronger antioxidative activity than an *ortho* analogue. This difference however would not be sufficient to explain the marked difference in the potency between ethyl *N*-pyridoxyl-*p*-aminobenzoate and ethyl *N*-vanillyl-*p*-aminobenzoate. Upon comparison of the effect of the trimethyl analogue of pyridoxine, 2,4,5-trimethyl-3-pyridinol (19), and the corresponding benzene derivative, 2,3,6-trimethylphenol in lard, it became clear that the absence or nearly absence of antioxidative activity in the pyridoxine derivatives would, at least in part, result from the nature of the pyridine ring (Figure 4).

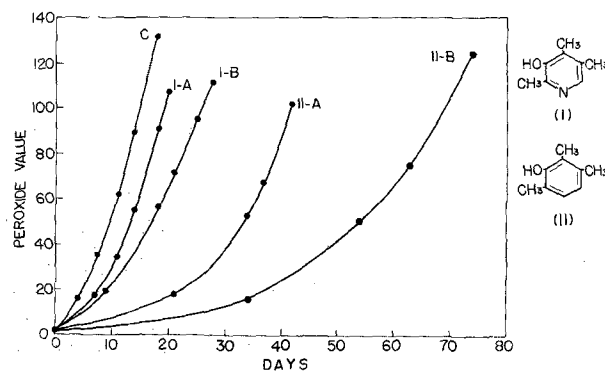


FIG. 4. Stability of lard containing (C) none, (I-A, I-B) 2,4,5-trimethyl-3-pyridinol (I) at a level of 1/3 and 1 μmole per gram of the substrate respectively, and (II-A, II-B) 2,3,6-trimethylphenol (II) at a level of 1/3 and 1 μmole per gram of the substrate, respectively. The test was conducted at 37°C., and the peroxide value was expressed as millimoles of peroxide per kilogram of fat.

Under the experimental conditions free vanillin failed to show antioxygenic potency. Conversion of vanillin to *N*-vanillyl- $\beta$ -phenylethylamine, ethyl *N*-vanillyl-*p*-aminobenzoate, vanillin isonicotinoylhydrazone, or the nicotinoylhydrazone eliminated the electron-withdrawing nature of the formyl group, thus these derivatives were active antioxidants (Figures 1, 2) (c.f. 20). The data presented by Bickoff indicate that the replacement of the formyl group of vanillin with a hydroxymethyl or a methyl group improved its antioxygenic nature for carotene (21). When ingested, ethyl *N*-vanillyl-*p*-aminobenzoate would probably be cleaved to vanillin and *p*-aminobenzoate *in vivo* (c.f. 6).

Although it is known that isonicotinoylhydrazone, salicylaldehyde isonicotinoylhydrazone, and pyridoxal isonicotinoylhydrazone form a chelate complex with a metallic ion, such as copper (8, 22, 23, 24), a lard which contained  $\text{Cu}^{++}$  and these compounds was even less stable than plain lard (Figure 2). The lard which contained 10 p.p.m. of  $\text{Cu}^{++}$  and the one with 10 p.p.m. of  $\text{Fe}^{+++}$  were unstable to an equal degree under the experimental conditions (Figure 2). When vanillin isonicotinoylhydrazone was added, the former became more stable than the latter. It was also found that vanillin isonicotinoylhydrazone reversed the effect of  $\text{Fe}^{+++}$  more efficiently than vanillin nicotinoylhydrazone. These results appear to indicate that various isonicotinoylhydrazones and nicotinoylhydrazones would form chelate complexes with the metallic ions of differing degrees of stability and that such chelate formation may play a part in their antioxidative mechanism in lard.

It thus becomes evident that an isonicotinoyl- or nicotinoylhydrazonomethyl group has two desirable properties as an *ortho* or *para* substituent for a phenolic antioxidant; one is the property to activate phenols, and the other is the property to combine with pro-oxidative metallic ions. An attempt was therefore made to synthesize 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde isonicotinoylhydrazone (BHB-INH), a structural analogue of butylated hydroxytoluene (BHT). The results proved that BHB-INH was an excellent antioxidant in the presence as well as in the absence of copper in lard (Figure 3). It was of interest to note that an equimolar mixture of BHT and BHB-INH behaved more like BHB-INH than BHT or an antioxidant of intermediate strength (Figure 3). Further study is necessary to demonstrate the possible synergism between BHT and BHB-INH conclusively.

The disadvantage of BHB-INH is its limited solubility in organic solvents such as ether, chloroform, dioxane, ethyl acetate, methanol, ethanol, and isopropylalcohol. BHB-INH is however soluble in pyridine. Replacement of an isonicotinyl group by a more lipophilic acyl radical may increase its solubility in fat since a general arrangement of "acyl-NH-N=CH-(phenol)" appears to be responsible for the activation of phenols. For chelate formation, an isonicotinoyl group would not be essential. The antioxidative potency of the resultant compound would however partially depend on the type of acyl groups combined (c.f. Figure 2).

Isonicotinoylhydrazone is used for the treatment of tuberculosis, and its toxicity has been reported. In a strain of mice the amount of free isonicotinoylhydrazone which is 100% lethal by oral administration is reported to be 300 mg./kg. (25). It is known that isonicotinoylhydrazone dissolved in glycerine or propyleneglycol and the hydrazone present in the form of a hydrazone are much less toxic (c.f. 25). Isonicotinoylhydrazone has also been reported to act as a physiological antioxidant in rats (26).

### Summary

Fat-soluble derivatives of vitamin B<sub>6</sub> including ethyl *N*-pyridoxyl-*p*-aminobenzoate, pyridoxal isonicotinoylhydrazone, and pyridoxine 5-monopalmitate showed little or no antioxidative activity for lard as well as for vitamin A at 37°C. The structurally related compounds, ethyl *N*-vanillyl-*p*-aminobenzoate, vanillin isonicotinoylhydrazone, and the nicotinoylhydrazone were found to be antioxidants although free vanillin was inactive. The removal of the electron-withdrawing nature at the formyl group in a vanillin molecule might be functional in the development of antioxygenic properties.

Evidence was obtained to show that an isonicotinoylhydrazonomethyl group not only activated a phenolic compound as an antioxidant but also removed pro-oxidative metallic ions from lard through chelation. For example, 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde isonicotinoylhydrazone (BHB-INH), a structural analogue of butylated hydroxytoluene (BHT), stabilized lard more efficiently than BHT on a molar basis in the presence as well as in the absence of  $\text{Cu}^{++}$ .

### REFERENCES

1. MacKenzie, D. G., "Biological Antioxidants, Fourth Conference," Josiah Macy Jr. Foundation, New York, 1949, p. 116.
2. Van Fleet, D. S., *Am. J. Botany*, **30**, 678 (1943).
3. Witting, L. A., Nishida, T., Johnson, O. C., and Kummerow, F. A., *J. Am. Oil Chemists' Soc.*, **34**, 421 (1957).
4. Hove, E. L., and Harris, P. I., *J. Nutrition*, **31**, 699 (1946).
5. Sakuragi, T., and Kummerow, F. A., *J. Am. Chem. Soc.*, **78**, 839 (1956).
6. Sakuragi, T., and Kummerow, F. A., *Arch. Biochem. and Biophys.*, **73**, 43 (1958).
7. Sah, P. P. T., *J. Am. Chem. Soc.*, **76**, 300 (1954).
8. Mauron, J., and Bujard, E., *Bull. Soc. Chim. Belg.*, **65**, 140 (1956).
9. Skita, A., and Stühmer, W., *Ger.*, **716**, 668, Dec. 24, 1941 (C.A. **38**, 2345<sup>b</sup>).
10. Sacconi, L., *J. Am. Chem. Soc.*, **75**, 5434 (1953).
11. Buu-Hoi, Ng. Ph., Xuong, Ng. D., Nam, Ng. H., Finon, F., and Royer, R., *J. Chem. Soc.*, **2953**, 1358.
12. Zubrys, A., and Siebenmann, C. D., *Can. J. Chem.*, **33**, 11 (1955).
13. Lundberg, W. O., and Chipault, J. R., *J. Am. Chem. Soc.*, **69**, 833 (1947).
14. Spruyt, J. P., *J. Am. Oil Chemists' Soc.*, **32**, 197 (1955).
15. Duff, J. C., *J. Chem. Soc.*, **1941**, 547.
16. Mowat, J. H., Bootle, J. H., Hutchings, B. L., Stockstad, E. L., Waller, C. W., Angir, R. B., Semb, J., Cosulich, D. B., and Subba Row, Y., *J. Am. Chem. Soc.*, **70**, 14 (1948).
17. Blair, J. A., *Biochem. J.*, **65**, 209 (1957).
18. Miller, G. J., and Quackenbush, F. W., *J. Am. Oil Chemists' Soc.*, **34**, 404 (1957).
19. Harris, S. A., *J. Am. Chem. Soc.*, **62**, 3203 (1940).
20. Ritter, D. M., *J. Am. Chem. Soc.*, **69**, 46 (1947).
21. Bickoff, E. M., *J. Am. Oil Chemists' Soc.*, **28**, 65 (1951).
22. Carl, E., and Marquardt, P., *Z. Naturforsch.*, **76**, 574 (1952).
23. Fallab, S., and Erlenmeyer, H., *Experientia*, **8**, 289 (1952).
24. Christensen, H. N., *J. Am. Chem. Soc.*, **79**, 4073 (1957).
25. Prescott, B., Kauffmann, G., and James, W. D., *Proc. Soc. Exptl. Biol. Med.*, **95**, 705 (1957).
26. Bercel, M., *Semana méd.*, **110**, **1**, 192, 206 (1957) (C.A. **51**, 8921<sup>a</sup>).
27. Cohen, L. A., *J. Org. Chem.*, **22**, 1333 (1957).

[Received January 9, 1958]