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THE REACTION OF L-ASCORBIC AND D-ISOASCORBIC ACID WITH NICOTINIC ACID AND ITS AMIDE

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L-Ascorbic acid, being a comparatively strong acid forms salts with organic amines such as Cinchona alkaloids (11), benzedrine (9), histidine (5), quinidine (3, 6), and aliphatic amines (2, 7, 10). All these compounds contain strongly basic amino groups. We became interested in the behavior of L-ascorbic acid toward weak cyclic organic bases while working on a synthesis of L-ascorbic acid by means of organic bases (12). These investigations were carried out during 1942. The results are published now because they extend the available knowledge in the literature.

It was found that L-ascorbic acid combines with nicotinamide to form a definite compound. Milhorat (8) described a color reaction of L-ascorbic acid and nicotinamide without isolating the compound. Its existence has since been reported by Bailey, Bright, and Jasper (1) and by Fox and Opferman (4), the former obtaining the compound by evaporation of solutions containing equimolecular amounts of the components, the latter by mixing the components in the dry state. Bailey, Bright, and Jasper also describe a compound consisting of nicotinic acid and L-ascorbic acid, made in a similar way. We prepared both compounds by crystallization from solvents. In addition we obtained a compound consisting of equimolecular amounts of D-isoascorbic acid and nicotinamide. The reactions between L-ascorbic acid and D-isoascorbic acid and these pyridine derivatives show some interesting features.

The reaction of L-ascorbic acid or D-isoascorbic with nicotinamide is a slow one. Its progress is visible because the newly formed compounds are yellow. The time required for the appearance of the yellow color varies with temperature, solvent etc., indicating that the reaction is not a mere salt formation which would take place immediately. In addition to the ordinary salt formation, there seems to be a further connection between L-ascorbic or D-isoascorbic acid and nicotinamide, probably of the nature of a secondary valence linkage. This assumption is supported by the optical properties of the new compounds. As is seen from Table I, the optical rotation of the complex differs considerably from that of the acid contained, with D-isoascorbic acid the rotation changing from levorotatory to dextrorotatory in the combination with nicotinamide.

Based on these findings we think it incorrect to name the new compounds "salts", and hence refer to the combination of L-ascorbic acid and nicotinamide as "nicotinamide-L-ascorbic acid complex" rather than calling it "nicotinamide L-ascorbate".

That mere salt formation is not sufficient explanation of the nature of the new compounds is still more evident from the behavior of L-ascorbic acid and D-isoascorbic acid toward free nicotinic acid. If free nicotinic acid possessing rather

weak basic properties is used as the base for "salt formation", only L-ascorbic acid reacts to form an addition product, whereas D-isoascorbic does not react. This is, to our knowledge, the sole reaction where L-ascorbic acid shows a distinct difference from D-isoascorbic acid in chemical behavior. The difference is so striking that it is useful for the differentiation between L-ascorbic and D-isoascorbic acid, and is suited for demonstration purposes. A 10–15% aqueous solution of L-ascorbic acid shaken for about 30 seconds with an equimolecular amount of nicotinic acid solidifies so that the flask can be turned upside down, whereas a corresponding solution of D-isoascorbic acid under the same conditions remains fluid, the nicotinic acid settles out, and is recovered unchanged by filtration.

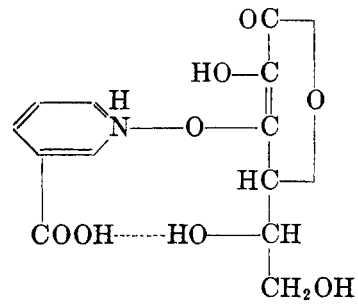
This difference in the formation of a complex cannot be explained by differences in acidity between L-ascorbic and D-isoascorbic acid, because both show practically the same *p*H in solutions. It seems logical to ascribe the difference in behavior to the difference in structure of both acids. Obviously the steric arrangement of the particular ascorbic acid is instrumental in bringing about the combination with nicotinic acid. This may be illustrated by a formula such as

TABLE I

OPTICAL ROTATION OF D-ISO- AND L-ASCORBIC ACID AND OF THEIR COMPLEXES WITH NICOTINAMIDE

COMPOUND	$[\alpha]_D^{25}$	DIFFERENCE
L-Ascorbic acid	+20.5°	6.6°
Nicotinamide-L-ascorbic acid	+27.1°	
D-Isoascorbic acid	−17.3°	30.4°
Nicotinamide-D-isoascorbic acid	+13.1°	

I for the nicotinic acid-L-ascorbic acid complex. The dotted line indicates a secondary valence linkage



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between both components in addition to the salt type connection. In D-isoascorbic acid the steric arrangement of the secondary hydroxyl group may be such as to preclude formation of a complex. The formula I is of course not proved, but this or a similar spatial arrangement is undoubtedly responsible for the observed differences.

In nicotinamide the carbamyl group may, in contrast to the free carboxylic group in nicotinic acid, still possess enough affinity to allow a combination.

The underlying mechanism of the reaction between L-ascorbic acid and nicotinic acid may in some way be connected with the mechanism of antiscorbutic activity. If, in order to exert Vitamin C properties, the ene-diolic acids have first to undergo a combination with proteins, such a mechanism would explain why L-ascorbic acid is strongly antiscorbutic, whereas D-isoascorbic acid, being less able to form complexes, has a much weaker activity. It would be interesting therefore to compare antiscorbutic activity of the known isomers of L-ascorbic acid with their capacity to form complexes with compounds such as nicotinic acid.

EXPERIMENTAL

The melting points were taken with an uncalibrated set of Anschütz thermometers.

I. Nicotinamide-L-ascorbic Acid Complex

A. In abs. alcohol. Finely powdered L-ascorbic acid (17.6 g.) is added to a solution of 12.2 g. of nicotinamide in 100 ml. of abs. alcohol at 20°. The mixture is shaken by hand. After about 2 to 3 minutes it turns yellow, and the contents solidify. The cake is broken up, and the yellow crystals are filtered, washed with about 30 cc. of cold alcohol, and dried; yield 26–28 g., m.p. 144–145°.

B. In dilute alcohol. A solution of 17.6 g. of L-ascorbic acid in 20 cc. of water is prepared by warming to 70–80°. To this solution is added in one portion a cold solution of 12.2 g. of nicotinamide in 250 cc. of abs. alcohol. The mixture turns yellow. It is cooled immediately to 20–25°, whereupon crystallization starts rapidly. After cooling to 0° for several hours, the crystals are filtered; yield 19 g., m.p. 145–146°.

C. In methanol. Nicotinamide (12.2 g.) and L-ascorbic acid (17.6 g.) are suspended in methanol (100 ml.). On warming on the steam-bath, crystals form after a few seconds. The mixture is cooled after about 10 minutes to room temperature, the crystals are filtered and washed with ice cold methanol; yield 21 g., m.p. 146–147°.

D. In water. Nicotinamide (12.2 g.) and L-ascorbic acid (17.7 g.) are dissolved in water (100 ml.) by warming to 50–60°. As soon as solution is complete the mixture is cooled with ice-water. The complex starts to crystallize immediately. After cooling in the refrigerator for several hours, the crystals are filtered, washed with abs. alcohol and dried; yield 16 g., m.p. 145–146°. From the mother liquor additional amounts are obtained by concentration *in vacuo* and addition of alcohol.

E. Properties of the compound. Solubility: in water 0° approx. 10%, 20° approx. 40%, 80° more than 100%; in abs. alcohol 20° approx. 2.4%, 80° approx. 8%; in methanol 0° approx. 5%, 20° approx. 10%, 60° approx. 20%; in acetone sparingly soluble; in ether practically insoluble; in benzene practically insoluble. *Optical rotation:* $[\alpha]_D^{19.5}$ +27.1° (water, c, 4.97); $[\alpha]_D^{20.5}$ +27.6° (water, c, 8.46). *Acidity:* *p*H, 3.93 (water, c, 8.46); *p*H, 3.94 (water, c, 4.97); *p*H, 3.91 (alcohol, c, 2.40). *Titration:* 0.1988 g. used 12.65 cc. 0.1 *N* iodine; Calc'd 13.35 cc. 0.1 *N* iodine.

Anal. Calc'd for $C_{12}H_{14}N_2O_7$: C, 48.32; H, 4.73; N, 9.39.
Found: C, 48.38; H, 4.56; N, 9.58.

II. Nicotinamide-D-isoascorbic Acid Complex

A. In alcohol. Finely powdered D-isoascorbic (35.2 g.) is dissolved at 70–80° in abs. alcohol (300 ml.). To the hot solution a solution of nicotinamide (24.4 g.) in abs. alcohol (100 ml.) is added in one portion. The mixture is cooled. Yellow crystals separate. They are filtered, washed with abs. alcohol, and dried; yield 35 g., m.p. 129°.

B. In dioxane. A solution of 12.2 g. of nicotinamide in 50 ml. of dioxane at about 70–

80° is added to a suspension of 17.6 g. of D-isoascorbic in 50 ml. of dioxane, warmed to about 60° on a steam-bath. Everything dissolves, and after cooling, crystals separate. They are filtered, washed with cold dioxane and dried; yield 24 g., m.p. 128–129°.

C. Properties. Optical rotation: $[\alpha]_D^{23}$ +13.10° (water, c, 6.99); *Acidity:* pH, 3.8 (water, c, 5.2588); *Titration:* 0.1966 g. used 12.95 cc. 0.1 *N* iodine; Calc'd for $C_{12}H_{14}N_2O_7$ 13.19 cc. 0.1 *N* iodine.

Anal. Calc'd for $C_{12}H_{14}N_2O_7$: C, 48.32; H, 4.73; N, 9.39.
Found: C, 48.74; H, 4.67; N, 9.69.

III. Nicotinic Acid-L-ascorbic Acid Complex

A. In water. To a solution of 17.6 g. of L-ascorbic acid in 100 ml. of water, 12.3 g. of finely powdered nicotinic acid is added. The mixture is shaken. After about one minute it turns yellow and soon solidifies. About one hour later the crystals are filtered and washed with a little ice-water; yield 22 g., m.p. 185°.

B. In methanol. A solution of 17.6 g. of L-ascorbic acid in 100 ml. of boiling methanol is added to a suspension of 12.3 g. of finely powdered nicotinic acid in 50 ml. of methanol. The mixture is warmed on the steam-bath. After a few minutes the color changes to yellow, and shortly thereafter, the mixture solidifies to a compact maze of crystals. They are filtered, washed with ice-cold methanol, and dried; yield 27 g., m.p. 182–183°.

C. Properties of the compound. The combination between L-ascorbic acid and nicotinic acid is a rather loose one, as is apparent from the following behavior of the complex. The compound is not very soluble in cold water. Ten grams does not dissolve readily on shaking in 100 ml. of water at 20°. On warming solution occurs, but simultaneously the yellow color disappears. If a hot 10% solution is allowed to cool to about 40°, nicotinic acid separates in colorless crystals. It can be isolated practically free of L-ascorbic acid by filtration. However, if the solution is allowed to stand in the refrigerator, the complex re-forms, visible by the reappearance of the bright yellow color. Filtration of this cooled solution gives the complex.

From this behavior it follows that at temperatures above about 40° the complex is split into the components.

Solubility: water 26°: 3.3% (colorless solution); abs. alcohol 25°: slightly soluble; acetone: practically insoluble; (for comparison: solubility of L-ascorbic acid in water 25°: 33%; of nicotinic acid in water 25°: 1.8%). *Optical rotation:* $[\alpha]_D^{23}$ + 10.8° (water, c, 1.39). *Acidity:* pH = 3.4 (c, 0.806). *Titrations: I* 0.0701 g. used 4.39 ml. 0.1 *N* iodine; Calc'd for $C_{12}H_{13}NO_8$ 4.20 ml. 0.1 *N* iodine. *II* 0.1390 g. used 9.02 ml. 0.1 *N* NaOH; Calc'd for $C_{12}H_{13}NO_8$ 8.77 ml. 0.1 *N* NaOH. *Dissociation constant:* K = 0.00002; (nicotinic acid: K = 0.000014).

Anal. Calc'd for $C_{12}H_{13}NO_8$: C, 48.16; H, 4.38; N, 4.68.
Found: C, 48.94; H, 4.44; N, 5.08.

IV. D-Isoascorbic Acid and Nicotinic Acid

If in the experiments described under IIIA and IIIB, the L-ascorbic acid is replaced by D-isoascorbic acid, no reaction takes place. When such mixtures are filtered, a practically quantitative recovery of nicotinic acid is obtained.

Acknowledgment. The microanalyses reported in this paper were carried out in our Microchemical Laboratory under the direction of Dr. Al Steyermark.

SUMMARY

L-Ascorbic acid and D-isoascorbic acid combine with nicotinamide to form definite compounds.

Nicotinic acid forms such a compound with L-ascorbic acid, but not with D-isoascorbic acid.

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REFERENCES

(1) BAILEY, BRIGHT, AND JASPER, *J. Am. Chem. Soc.*, **67**, 1184 (1945).
(2) CHRISTIANSEN, U. S. Patent 2,213,977 (Sept. 10, 1940).
(3) EISENBRAND AND SIENZ, U. S. Patent 2,140,989 (Dec. 20, 1938).
(4) FOX AND OPFERMAN, U. S. Patent 2,433,688 (Dec. 30, 1947).
(5) HOFFMANN-LA ROCHE AND CO., A.-G., Germ. Patent 718,991 (March 5, 1942).
(6) HOFFMANN-LA ROCHE AND CO., A.-G., Swiss Patent 214,109 (July 1, 1941).
(7) LAUTENSCHLÄGER AND LINDNER, U. S. Patent 2,249,903 (July 22, 1941).
(8) MILHORAT, *Proc. Soc. Exp. Biol. and Med.*, **55**, 52 (1944).
(9) RUSKIN, U. S. Patent 2,294,937 (Sept. 8, 1942).
(10) VOLWILER, U. S. Patent 2,132,662 (Oct. 11, 1938).
(11) WARNAT, U. S. Patent 2,288,965 (July 7, 1942).
(12) WENNER, U. S. Patent 2,443,487 (June 15, 1948).