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The Biological Potency of the Natural Tocopherols and Certain Derivatives

BY MILTON JOFFE AND PHILIP L. HARRIS

The recent availability of the pure α -, β - and γ -tocopherols, α -tocopheryl acid succinate, β -tocopheryl azobenzene-4-carboxylate, and γ -tocopheryl palmitate¹ permitted a determination of their relative biological potencies for the first time. Bacharach² has conducted a preliminary assay on natural α - and β -tocopherols with inconclusive results. He covered the literature on this subject very thoroughly through 1938 and concluded that no adequate relative biological potencies could yet be assigned to the various tocopherols. Since then, Mason³ has mentioned that in his laboratory preliminary assays have indicated relative biopotencies for α , β and γ of $1-1/2-1/4$, respectively. Also, Karrer, *et al.*,⁴ using synthetic materials, have reported that β -tocopherol is $1/2$ to $1/3$ and gamma tocopherol $1/3$ to $1/8$ the potency of α -tocopherol.

Experimental

The experimental bioassay procedure used in this Laboratory follows the rat-antisterility test of Evans and Burr⁵ as modified by Mason.⁶ Young female rats from our breeding colony are used if their body weight is 35 g. or more when weaned at twenty days of age. They are placed on our Diet No. 30, very deficient in tocopherol content, and maintained until they weigh 150 g. Diet No. 30 contains 20% crude casein, 27% Cerelose, 27% corn starch, 4% U. S. P. No. 2 salt mixture, 10% dried brewer's yeast, and 12% lard containing vitamin A and vitamin D concentrates to furnish 12 U. S. P. units of vitamin A and 5 U. S. P. units of vitamin D₂ per gram of diet. The use of this diet over a period of several years has invariably resulted in 100% resorption gestations in negative control animals. Experimental groups are made up isogenetically and the animals mated with normal, proved males. The vitamin E supplements are fed on the fourth, fifth and sixth days after conception. Calibrated droppers are used to administer the vitamin E test substances which are diluted in tocopherol-free olive oil. On the sixteenth day of pregnancy, the rats are laparotomized and a record made of the number of living, dead and resorbed fetuses. All animals having two or more living young, providing that at least four implantation sites are present, are considered as responding positively. In each group, the proportion of positive re-

sponses to total responses represents the litter efficiency induced by the vitamin E supplement fed to that group. For intergroup comparisons, the most accurate response is at a 50% level. In fact, the potency of a substance is usually expressed as the number of milligrams (M.F.D. or Median Fertility Dose) required to produce a "litter efficiency" of 50%.

Experimental Results

In Table I are given the results obtained in a single typical assay on crystalline α -tocopheryl acid succinate. From these data, a dose-response

TABLE I
THE VITAMIN E EFFECTIVENESS OF CRYSTALLINE α -
TOCOPHERYL SUCCINATE

Amount fed as tocopherol, mg.	Response		
	Positive	Negative	Litter efficiency, %
0.6	2	14	12.5
0.85	4	9	30.8
1.2	7	4	63.6
1.7	14	0	100.0

curve can be calculated according to the method of Bliss⁷ from which the M.F.D. of α -tocopherol as the succinic acid ester may be determined. The slope of this particular curve, relating litter efficiency [expressed as probits⁸] to the logarithm of daily dose, equals 4.85. This value agrees very well with the average figure obtained, 4.989, in the collaborative study reported by Hume,⁹ upon which the International Standard Unit for vitamin E is based. It is interesting that all of our values for α -tocopherol and α -tocopherol derivatives have a dose-response curve with a slope equal to or greater than that observed in this assay. However, for the β -tocopherols, a flatter curve has been invariably obtained and for the γ -tocopherols, a still flatter curve has been found. This will be discussed separately in greater detail when the relative potencies of the synthetic and natural tocopherols are reported.

It may be noted that the M.F.D. for α -tocopherol in this example would be approximately 1.0 mg. From assay to assay, this absolute value varies somewhat, from 0.6-1.0, as might be expected. It merely emphasizes the fact that a vitamin E reference standard must be included in each assay.

(1) J. G. Baxter, C. D. Robeson, J. D. Taylor and R. W. Lehman, *THIS JOURNAL*, **65**, 918 (1943).

(2) A. L. Bacharach, *Biochem. J.*, **32**, 2017 (1938).

(3) K. E. Mason, *Yale Journal of Biol. Med.*, **14**, 605 (1942).

(4) P. Karrer, H. Koenig, B. H. Ringier and H. Salomon, *Helv. Chim. Acta*, **22**, 1139 (1939).

(5) H. M. Evans and G. O. Burr, *Mem. Univ. Calif.*, (1927).

(6) K. E. Mason, *J. Nutrition*, **23**, 59 (1942).

(7) C. I. Bliss, *Ann. Applied Biol.*, **22**, 134 (1935).

(8) C. I. Bliss, *Ind. Eng. Chem., Anal. Ed.*, **13**, 84 (1941).

(9) E. M. Hume, *Nature*, **148**, 472 (1942).

TABLE II
RELATIVE BIOLOGICAL POTENCIES OF THE NATURAL TOCOPHEROLS AND DERIVATIVES

Substance fed	Amount fed as tocopherol, mg.	No. of rats	Litter efficiency, %	Calculated M.F.D.	Dose equivalent to 1 mg. of natural α -tocopherol	Relative potencies
α -Tocopherol	0.75	9	55.6	0.75	1	1.0
	1.25	8	100.0			
α -Tocopheryl acid succinate	0.75	18	55.6	0.7	1	1.0
	0.90	6	83.3			
β -Tocopherol	1.5	6	33.3	1.9	2.5	0.4
	2.0	4	50.0			
	3.0	5	80.0			
	6.0	4	100.0			
β -Tocopheryl azobenzene-4-carboxylate	3.0	5	0	approx. 6.0	8	.125
	9.0	3	100.0			
γ -Tocopherol	2.0	4	0	8.9	12	.083
	4.0	5	20			
	8.0	6	33.3			
	8.9	10	50.0			
	10.0	9	100.0			
γ -Tocopheryl palmitate	2.0	6	0	9.0	12	.083
	6.0	11	9.1			
	7.5	7	0			
	9.0	8	50.0			
Synthetic <i>dl</i> - α -tocopherol ^a	1.0	10	50.0	1.0	1.3	.75

^a This material was included in the assay to serve as a standard for comparison between laboratories since it has been widely available for several years. The greater potency of the natural tocopherol over the synthetic *dl*-form, shown in this comparison, will be discussed in a later publication.

Comparing the M.F.D. for each of the pure compounds available for this study, the values in Table II are obtained.

The α - and γ -tocopherol esters have essentially the same potency as their respective free forms, whereas the β -tocopherol ester is definitely less potent than might be expected considering its content of β -tocopherol. In our earlier bioassays, comparing the biopotency of α -tocopherol with a variety of its esters, we obtained results indicating that the esters were more potent than equivalent amounts of the free tocopherol. Similar findings were reported by Demole, *et al.*,¹⁰ in 1939. However, upon repeatedly testing this point, we now feel that relatively greater stability of the tocopherol esters probably accounted for their apparently superior potency over the free α -tocopherol in our earlier tests. Actually, when extreme care is taken to prevent any destruction of tocopherol up to and during the feeding of it, its biopotency is found to be the same as that of equivalent quantities of its esters.

In all probability, the disparity in our results and those previously reported in the literature for the biopotency of γ -tocopherol may be due to the

relatively greater purity of our compound. Some of the first samples of supposedly pure γ -tocopherol received by us for test showed a biopotency of $1/2-1/3$ that of α -tocopherol. Later, when more precise chemical tests for differentiating the various tocopherols in admixtures were developed,¹¹ it was found that sufficient α -tocopherol had been present as a contaminant to give these results. Later samples, of 100% purity, are those used in the present assay.

Summary

The vitamin E potency of pure natural α -, β - and γ -tocopherols, crystalline α -tocopheryl acid succinate, β -tocopheryl azobenzene-4-carboxylate, and γ -tocopheryl palmitate has been determined by recognized bioassay procedures.

Natural α -tocopherol and its succinic acid ester (equivalent quantities of tocopherol) have essentially the same M.F.D., *i. e.*, 0.75 mg. Pure natural β -tocopherol has an M.F.D. of 1.9 mg., approximately 0.4 as potent as the α -form. The β -tocopherol in the form of its azobenzene-4-carboxylate ester is still less potent with an M.F.D. of approximately 4.0 mg. Natural γ -tocopherol, both as the free alcohol and as the palmitate, has

(10) V. Demole, O. Isler, B. H. Ringier, H. Salomon and P. Karrer, *Helv. Chim. Acta*, **22**, 65 (1939).

(11) J. B. Baxter, J. D. Taylor and K. S. French, to be published.

a m.f.d. of 9.0 mg. Consequently, this form of vitamin E is only one-twelfth as potent, measured by the rat-antisterility test, as α -tocopherol and only about one-fifth as potent as β -tocopherol.

Crystalline α -tocopheryl acid succinate is suggested as a Standard of Reference for vitamin E.

The present International Standard, *dl*- α -tocopheryl acetate, recently proposed,⁹ is a viscous, oily compound relatively more difficult to purify, duplicate and handle than the crystalline succinic acid ester.¹

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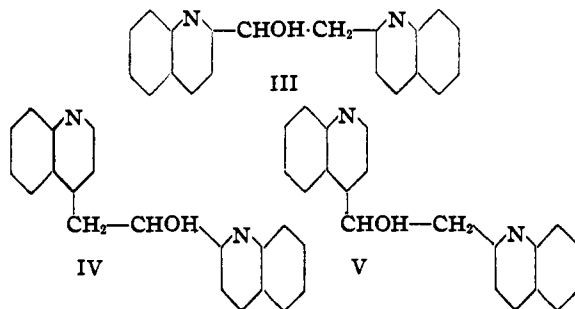
[CONTRIBUTION FROM NICHOLS LABORATORY, NEW YORK UNIVERSITY]

Condensation Reactions of Cinchoninaldehyde and Quinaldaldehyde¹

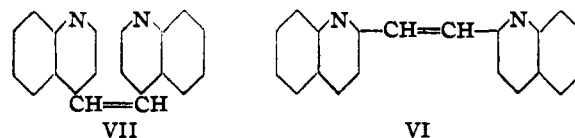
BY HARRY KAPLAN² AND H. G. LINDWALL

Earlier studies³ of quinoline-4-aldehyde (cinchoninaldehyde II) and quinoline-2-aldehyde (quinaldaldehyde I) have been extended by investigations of further condensation reactions.

Quinaldaldehyde (I) has been found to condense with quinaldine and also with lepidine in aqueous alcoholic medium with or without diethylamine as a catalyst. In this way, 1,2-bis-(quinolyl-2)-ethanol-1 (III) and 1-(quinolyl-2)-2-(quinolyl-4)-ethanol-1 (IV) have been obtained, respectively. Under similar conditions, cinchoninaldehyde (II) has been found to yield an aldol-like product with quinaldine, namely, 1-(quinolyl-4)-2-(quinolyl-2)-ethanol-1 (V). The analogous product from II and lepidine could not be obtained.



Lepidine and cinchoninaldehyde heated together in a sealed tube with glacial acetic acid and acetic anhydride, or with anhydrous zinc chloride, gave 1,2-bis-(quinolyl-4)-ethene (VII). Quinaldine and quinaldaldehyde (I) similarly yielded 1,2-bis-(quinolyl-2)-ethene (VI); com-



(1) Original manuscript received February 2, 1942.

(2) Present address: The Winthrop Chemical Co., Albany, N. Y.

(3) (a) Ewartler and Lindwall, *THIS JOURNAL*, **59**, 524 (1937);

(b) Kaplan, *ibid.*, **63**, 2654 (1941).

ound VI was also obtained by the dehydration of compound III through the use of hot glacial acetic acid and acetic anhydride.

Both cinchoninaldehyde (II) and quinaldaldehyde (I) formed anils with sulfanilamide, compound IX from II and VIII from I. Also the respective carbazones of II and I were prepared.

Experimental

1,2-Bis-(quinolyl-2)-ethanol-1 (III).—Quinaldine (0.5 cc.) and quinaldaldehyde (0.5 g.) were dissolved in aqueous alcohol (10 cc. 80% alcohol), with or without 6 drops of diethylamine. After refluxing about 6 hours, III separated from the hot solution; yield 0.75 g. (75%). Recrystallized from ethanol, methanol, or pyridine; m. p. 167–168°.

Anal. Calcd. for $C_{20}H_{18}ON_2$: N, 9.33. Found: N, 9.13.

1-(Quinolyl-2)-2-(quinolyl-4)-ethanol-1 (IV).—A solution of quinaldaldehyde (1 g.) and lepidine (1 cc.) in 15 cc. of aqueous alcohol (80%) with or without 6 drops of diethylamine, was refluxed about six hours. After removal of solvent by distillation under reduced pressure, the residue was recrystallized from pyridine and then from ethanol as practically colorless small flaky crystals; yield, 1.46 g. (73%); m. p. 191–192°.

Anal. Calcd. for $C_{30}H_{18}ON_2$: N, 9.33. Found: N, 9.23.

1-(Quinolyl-4)-2-(quinolyl-2)-ethanol-1 (V).—Cinchoninaldehyde (1 g.) and quinaldine (1 cc.) were dissolved in 2 cc. *n*-propanol, with or without 6 drops of diethylamine, and the reaction mixture was kept at reflux overnight. V precipitated from the reaction mixture, and was recrystallized from ethanol or *n*-propanol; yield 1.44 g. (72%); m. p. 180–182°.

Anal. Calcd. for $C_{30}H_{18}N_2O$: C, 80.0; H, 5.33. Found: C, 79.9; H, 5.16.

Benzoate of V (Va).—V (60 mg.) was dissolved in pyridine (1.5 cc.) and benzoyl chloride (0.2 cc.) was added, as well as sufficient chloroform (0.8 to 1.0 cc.) to dissolve the resultant precipitate. After standing overnight, the reaction mixture was poured into a small amount of dilute sulfuric acid. Va separated as a solid and was recrystal-