187. THE BIOLOGICAL ACTIVITY OF THE OXIDATION PRODUCTS OF α -TOCOPHEROL

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THE appearance of the results reported by Golumbic & Mattill [1940] has clarified to some extent the position regarding the biological activity of the oxidation products of α -tocopherol. The major discrepancy concerned the yellow *p*-tocoquinone which Karrer *et al.* [1938], John *et al.* [1939], Wright & Drummond [1940], Karrer & Geiger [1940] and Golumbic [1940] had reported as being unable to replace vitamin E in rat experiments, whereas, in strong contrast, Emerson *et al.* [1939] and Evans *et al.* [1939] maintained that its activity was little less than that of α -tocopherol.

Golumbic & Mattill [1940] now suggest that the oxidation resulting from the reagent employed by Emerson *et al.* [1939], namely, FeCl_3 acting in methyl alcohol at room temperature, was incomplete and that the activity of their preparation in biological tests was due to residual α -tocopherol. However, this explanation fails to account for the approximately equal biological activities shown by their preparation of the quinone and the parent substance.

The spectroscopic data given for the sample of the α -tocoquinone examined by Emerson *et al.* [1939] clearly show that if there was unchanged α -tocopherol present the amount was probably less than 10 or 15 %.

We have repeated this oxidation, following as closely as possible the directions given by Emerson and his colleagues. 250 mg. were allowed to react with 1.25 g. FeCl_3 in 12.5 ml. methyl alcohol for 90 min. at room temperature. The quinone, separated by diluting the reaction mixture with water and extracting with ether, was a yellow oil showing on spectroscopic examination selective absorption at 262 and 268 $\text{m}\mu$. At both wave-lengths the extinction coefficient was the same, $E_{1\text{ cm.}}^{1\%} = 375$. The value deduced from the data given in the paper by Emerson *et al.* [1939] is $E_{1\text{ cm.}}^{1\%} = 310$.

Our quinone preparation was inactive in doses of 5 mg. when tested on rats (see B, Table 1). We are satisfied, therefore, that this oxidation product does not possess a biological activity of the same order as the parent alcohol. Moreover, our spectroscopic data support the view of Golumbic & Mattill [1940] that the preparation examined by Emerson *et al.* [1939] was incompletely oxidized. It still remains difficult to account for the equal activities recorded by these workers.

Oxidation with a warm methyl alcoholic solution of AgNO_3 , as employed by Cuthbertson *et al.* [1940], gives the same quinone. The product is spectroscopically identical with that obtained by cold FeCl_3 and it is also biologically inactive (see C, Table 1).

More vigorous oxidation with AgNO_3 , or with HNO_3 , yields a red compound which appears to have the structure of an *o*-quinone [Smith *et al.* 1939].

Table 1

Dose	Oxidizing agent	Rat no.	Uterine index
A	—	3	$(9.49 + 10)/5 = 3.90$
		7	$(8.42 + 9)/5 = 3.48$
		34	$(6.35 + 6)/5 = 2.47$
		16	$(9.10 + 8)/5 = 3.42$
		39	$(6.32 + 7)/5 = 2.66$
			Mean = 3.19
B	FeCl ₃	9	$(1.00 + 0)/5 = 0.20$
		23	$(0.90 + 0)/5 = 0.18$
		37	$(1.14 + 0)/5 = 0.23$
		26	$(5.76 + 8)/5 = 2.75$
		28	$(4.07 + 0)/5 = 0.81$
		14	$(1.69 + 0)/5 = 0.34$
			Mean = 0.75
C	AgNO ₃	4	$(1.30 + 0)/5 = 0.26$
		19	$(0.89 + 0)/5 = 0.18$
		20	$(2.65 + 0)/5 = 0.53$
		35	$(1.30 + 0)/5 = 0.26$
		12	$(0.67 + 1)/5 = 0.33$
		29	$(1.86 + 0)/5 = 0.37$
		30	$(4.48 + 0)/5 = 0.90$
		45	$(6.75 + 8)/5 = 2.95$
			Mean = 0.72
D	HNO ₃	49	$(1.47 + 0)/5 = 0.29$
		6	$(2.73 + 3)/5 = 1.15$
		17	$(6.02 + 8)/5 = 2.80$
		25	$(7.09 + 8)/5 = 3.02$
		36	$(1.04 + 0)/5 = 0.21$
		13	$(2.33 + 0)/5 = 0.47$
			Mean = 1.32

We have prepared this compound. It showed no selective absorption in the ultraviolet, but there was a band with poor persistence at about 405 m μ . Doses of 5 mg. gave a positive response in rat tests (see D, Table 1), but the activity was less than that of the parent compound.

The biological tests were carried out according to the technique devised by Mason [1939] who defines the Uterine Index by the formula:

$$\frac{1}{5} (\text{Wt. of uterine contents} + \text{No. of viable fetuses (in g.)}).$$

Values less than 0.35 indicate "completely negative" responses; those greater than 0.35 but less than 1.0 indicate "subminimal", and those greater than 1.0 "positive" responses. α -Tocopherol acetate and the *o*-quinone give positive responses, but the FeCl₃ and AgNO₃ oxidation products show subminimal activity.

In view of the activity of the *o*-quinone, attempts were made to reduce this compound to the corresponding quinol. So far they have been unsuccessful, in that the reduced compound has not been isolated in a stable form. By reduction with H₂ in the presence of Raney nickel, a pale yellow product was obtained showing a broad band at 262 m μ , but its unstable character has so far been an obstacle to satisfactory isolation.¹

Our investigation of these oxidation products had as its main object the further examination of the hypothesis that the tocopherols and certain of their derivatives act as oxidation-reduction systems in the tissues [Drummond, 1939].

¹ Unfortunately this work has been temporarily suspended owing to war conditions.

The inactivity of the *p*-quinone, now amply confirmed, seems to dispose of the idea that the quinone-quinol, $I \rightleftharpoons II$ change occurs in the tissues. This has led Golumbic & Mattill [1940] to postulate that the free phenoxyl radical, III, may be the biologically important unit of a reversible system.

The fairly high biological activity of the product of oxidation by HNO_3 , which the work of Smith *et al.* [1939] suggests may have the constitution IV, is

Fig. 1.

doubtless related to the preservation of certain features of the structure of tocopherol, i.e. the integrity of the oxygen ring and the phytyl side-chain.

In view of the fact that storage of the tocopherols can take place in the body fat and certain other tissues of rats after the administration of relatively large doses, it was thought to be of interest to see whether this active quinone would also be retained.

A group of 4 adult female rats, all of which had been deprived of vitamin E for at least 10 months, were given orally a dose of 10 mg. of the *o*-quinone on each of 5 successive days. For purposes of comparison, a similar group of rats was employed for experiments in which (a) α -tocopherol, (b) the *p*-quinone prepared by oxidation with FeCl_3 and (c) the *p*-quinone prepared by AgNO_3 oxidation were administered.

The body fats and liver fats of these animals were examined by the method described by Cuthbertson *et al.* [1940]. The results with the liver fats were unsatisfactory owing to interference by the large amounts of vitamin A present. None of the body fats showed any evidence of selective absorption on spectroscopic examination, but that from the group to which α -tocopherol acetate had been given yielded unsaponifiable fractions which darkened appreciably on oxidizing with AgNO_3 .

Another experiment of a similar nature was made in which a single dose of 10 mg. of each of the four compounds was given. A week later, the body fats were removed and the unsaponifiable fractions were examined. The only positive result was in the case of those obtained from the animals which had had 10 mg. each of the *o*-quinone. The unsaponifiable fraction showed the triple band illustrated in Fig. 1. Oxidation with AgNO_3 , as practised in examining material for tocopherol, did not alter the character of the absorption.

SUMMARY

1. The products formed by the oxidation of α -tocopherol with AgNO_3 and FeCl_3 are spectroscopically identical. Both show subminimal activity in rat tests when given in oral doses of 5 mg. We cannot confirm the finding of Emerson *et al.* [1939] that α -tocoquinone prepared in this manner has substantially the same activity as the parent compound.

2. It does not appear that any of the known oxidation products of α -tocopherol are likely to take part in a reversible oxidation system in the body. The biological activity of the red *o*-quinone obtained by oxidizing α -tocopherol with nitric acid is relatively high, but this is probably attributable to the integrity of the oxygen ring and the phytyl side-chain. The only plausible suggestion regarding the nature of reversible systems affecting the oxidation of vitamin E is that of Golumbic & Mattill [1940] which relates α -tocopherol to the corresponding free phenoxyl radical.

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