Biological potencies of ϵ- and ζ_1-tocopherol and 5-methyltocol

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It is now clear that the tocopherols found in nature include two distinct series of compounds. The first, and until recently the only known, series consists of methylated derivatives of tocol (2-methyl-2-(4′,8′,12′-trimethyltridecyl)-6-chromanol). The second consists of compounds related to the tocols, but containing a trimethyltrideca-3,7,11-trienyl side-chain. The two series are thus related (as are the vitamin K_1 and vitamin K_2 series) by substitution of unsaturated for saturated isoprenoid units in the side-chain. We suggest the trivial name 'tocotrienol' for 2-methyl-2-(4′,8′,12′-trimethyltrideca-3,7,11-trienyl)-6-chromanol, the unsaturated derivative of tocol, and shall here designate the members of the new series as methylated tocotrienols. The natural ϵ-tocopherol of wheat has been shown by Green, Mamalis, Marcinkiewicz & McHale (1960) not to be 5-methyltocol, with which it had been previously identified (Eggitt & Ward, 1953), but to be 5,8-dimethyltocotrienol, the unsaturated derivative of β-tocopherol. The position of ζ-tocopherol is less clear. The name was first given to a tocopherol shown to be present in wheat, barley and rye by Green, Marcinkiewicz & Watt (1955) and was identified at that time with 5,7-dimethyltocol. Later, Green & Marcinkiewicz (1956) gave the same name to a tocopherol in rice. However, Green, McHale, Marcinkiewicz, Mamalis & Watt (1959) showed that the two substances were different. The substance in wheat, ζ_1-tocopherol, is in fact 5,7,8-trimethyltocotrienol (the unsaturated derivative of α-tocopherol), whereas the substance in rice, ζ_2-tocopherol, is the authentic 5,7-dimethyltocol. 5-Methyltocol itself has so far not been found in a natural product, but it has been synthesized by McHale, Mamalis, Marcinkiewicz & Green (1959) and Green *et al.* (1959).

Although the biological potency of what is now known to be ζ_2-tocopherol was determined by gestation–resorption assay (Bunyan, Green, Mamalis & Marcinkiewicz, 1957), it is more important to know the potency of ζ_1-tocopherol, since this is the tocopherol found in wheat (and probably in barley and rye). Although Ward (1958) estimated the potency of ϵ-tocopherol by its activity in preventing testicular degeneration and uterine pigmentation in the rat, it was considered desirable to determine the potency of this substance by the conventional gestation–resorption assay. At the same time the biological potency of 5-methyltocol was of interest, since this is the remaining member of the tocol series. All three substances were assayed in one test.

EXPERIMENTAL

Preparation of compounds

ϵ- and ζ_1-Tocopherol. The tocopherols were isolated from wheat-bran oil by methods that will be described in detail elsewhere. Their identity and purity were checked by ultraviolet and infrared spectroscopy and by two-dimensional paper chromatography. ϵ-Tocopherol was obtained nearly pure, but ζ_1-tocopherol was obtained only in the form of a concentrate, though free of other tocopherols or reducing impurities. Each specimen used for biological assay was separately assayed chemically for tocopherol content, and this measurement was used for the final calculation of biological potency.

5-Methyltocol. The racemic form of this compound was synthesized from tocol by the method of Green *et al.* (1959).

Biological tests

Female rats of the Norwegian hooded strain were reared on a vitamin E-free diet of the percentage composition: casein (Low Vitamin Content, Genatosan Ltd) 25, sucrose 50, dried brewer's yeast 10, lard 10, McCollum's salt mixture 5, with the addition of 40 i.u. vitamin A and 2·5 i.u. vitamin D_3/g diet. Gestation–resorption assays of the three substances compared with DL-α-tocopheryl acetate were carried out by the technique previously described (Bunyan *et al.* 1957).

RESULTS

Owing to the low potency of the test substances and to the relatively small amounts available, it was possible to use only seven to ten rats on each substance, so that, although linearity and parallelism were satisfactory, the slopes of the probit–log (dose) lines were not significant. The evidence about the slope from previous assays was used, provided that it proved concordant with the new results, which it did. The

Table 1. *Biological potencies of ζ_1- and ϵ-tocopherol and 5-methyltocol assessed by gestation–resorption tests in the rat*

Substance	No. of rats	Potency relative to DL-α-tocopheryl acetate* (%)	Limits of error ($P = 0{\cdot}95$) (%)
D-ζ_1-Tocopherol	10	32	13–82
D-ϵ-Tocopherol	7	5	2–16
DL-5-Methyltocol	9	10	4–25

* A pooled estimate of slope from previous assays was used in the calculation of potency and limits of error (see p. 256).

available evidence was derived from the duplicate assays of 5,7-dimethyltocol (Bunyan *et al.* 1957) and from the assay of η-tocopherol (Bunyan, 1958). These three estimates of slope proved to be concordant with the estimates in the new assays (χ^2 with 9 degrees of freedom = 3·68) and the pooled slope was significant at $P < 0{\cdot}001$.

Table 2. *Relative potencies of tocopherols in various biological tests*

Substance	With rats: G (1, 2, 3)	G (4, 5, 6)	W (7)	U (8)	T (8, 9*)	L (10)	L (11)	F (12)	Ha (13)	Ha (14)	Ha (15)	Hb (13)	Hb (15)	R (16)	R (17)	With rabbits C (18)	With chickens L (19)	With chickens E (20)
DL-α-Tocopheryl acetate	**91**	—	**100**	—	—	—	114	—	**91**	**98†**	—	—	—	—	—	—	**100**	—
DL-α-Tocopherol (DL-5,7,8-trimethyltocol)	100 (1)	—	100	—	—	**100**	100	**100**	—	100	**100**	**100**	**100**	**100**	**100**	100	100	—
D-α-Tocopheryl acetate	124 (1)	—	—	—	—	—	232	—	—	144	—	—	—	—	—	—	134	—
D-α-Tocopherol	135 (1)	**135**	—	**135**	**135**	—	**232**	—	—	130	130	—	100	—	109	**112**	134	**135**
D-α-Tocopheryl succinate	—	—	—	—	—	—	—	—	—	112	—	—	—	—	—	—	—	—
D-α-Tocopheryl polyethylene glycol 1000-succinate	—	—	—	—	—	—	—	—	—	—	—	—	—	—	277	—	—	—
DL-β-Tocopherol (DL-5,8-dimethyltocol)	—	—	25	—	—	—	—	74	25	—	—	54	—	55	—	—	—	—
D-β-Tocopherol	—	54 (4)	—	—	—	—	—	—	—	—	20	—	40	—	36	34	—	—
DL-γ-Tocopherol (DL-7,8-dimethyltocol)	—	—	19	—	—	—	—	17	18	—	—	67	—	5	—	7	—	—
D-γ-Tocopheryl acetate	—	—	—	—	—	—	—	—	—	19	—	—	—	—	—	—	—	—
D-γ-Tocopherol	—	11 (4) 1 (6)	—	—	13 (9)	8	6	—	—	22	4	—	30	—	83	22‡	—	34
DL-δ-Tocopherol (DL-8-methyltocol)	—	—	—	—	—	—	—	—	0·3	—	—	26	—	4	—	—	—	—
D-δ-Tocopherol	—	1 (5)	—	—	—	—	—	—	—	—	3	—	20	—	17	—	—	13
D-ε-Tocopherol	5§	—	—	< 10	< 10 (8)	—	—	—	1–5	—	—	133	—	23	—	—	—	—
DL-5-Methyltocol	9§	—	—	—	—	—	—	—	3–13	—	—	67	—	7	—	—	—	—
D-ζ_1-Tocopherol	29§	—	—	—	—	—	—	—	23	—	—	106	—	—	—	—	—	—
DL-5,7-Dimethyltocyl acetate	47 (2)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
DL-5,7-Dimethyltocol	—	—	—	54	54 (8)	50	—	—	60	—	—	121	—	60	—	—	—	—
DL-η-Tocopherol (DL-7-methyltocol)	3 (3)	—	—	—	—	—	—	—	1	—	—	88	—	0·4	—	—	—	—
DL-Tocol	—	—	—	—	—	—	—	—	1–5	—	—	34	—	1	—	—	—	—

G, gestation–resorption; W, weight gain during gestation; U, uterine pigmentation; T, testicular degeneration; L, liver storage; F, deposition in body-fat; H, dialuric acid-induced erythrocyte haemolysis (a, after in vivo dosing; b, after in vitro dosing); R, reversal of respiratory decline in liver slices; C, cure of creatinuria; E, deposition in eggs.

Potencies are stated relative to DL-α-tocopherol = 100, for ease of comparison; but the value for the substance used as a standard is given in bold figures in each column. Where DL-α-tocopherol was not tested, the standard substance is assigned the potency found in the gestation–resorption assay. References are given in parentheses.

* Other criteria also used.
† Relative potencies based on tocopherol content only.
‡ Probably contaminated with α-.
§ This study.

(1) National Formulary X (1955).
(2) Bunyan *et al.* (1957).
(3) Bunyan (1958).
(4) Joffe & Harris (1943).
(5) Stern, Robeson, Weisler & Baxter (1947).
(6) Weisler, Baxter & Ludwig (1945).
(7) Gottlieb, Quackenbush & Steenbock (1943).
(8) Ward (1958).
(9) Filer, Rumery & Mason (1946).
(10) Bolliger & Bolliger-Quaife (1956).
(11) Quaife (1952).
(12) Lundberg, Barnes, Clausen, Larson & Burr (1947).
(13) Bunyan, Green, Edwin & Diplock (1960).
(14) Friedman, Weiss, Wherry & Kline (1958).
(15) Rose & György (1952).
(16) Green, Edwin, Bunyan & Diplock (1960).
(17) Rodnan, Chernick & Schwarz (1956).
(18) Hove & Harris (1947).
(19) Pudelkiewicz *et al.* (1960).
(20) Dju, Quaife & Harris (1950).

Using the pooled data, we then completed the calculations by the method described by Finney (1952), giving first approximations to the relative potencies and limits of error as shown in Table 1.

DISCUSSION

With the completion of these results, all the tocopherols derived from tocol have now been assayed for vitamin E activity by the standard gestation–resorption test. It is convenient at this point to assemble the values so far obtained in the comparative assays of tocols and tocotrienols by various methods, which has been done in Table 2. Ames (1956) gave the relative potencies of α-, β-, γ- and δ-tocopherols as 100:33:1:<1 by the gestation–resorption assay, and Griffiths (1959) found liver storage of these substances by the depleted chick to be in the ratios 100:41:20:0; however, neither author stated whether D or DL compounds were used, and their results have therefore not been included in the table.

The results of tests involving oral dosing give roughly similar results, suggesting that the general order of potency is trimethyl- > dimethyl- > monomethyl-tocol, with the unsaturated derivatives of α- and β-tocopherol (ζ_1- and ϵ-tocopherol) less active than the corresponding saturated compounds.

Only rarely have direct comparisons between DL and D forms of the tocopherols been made. There appears to be some conflict of opinion, but there is no doubt that the D form is more active than the DL form, although estimates of the difference vary. Thus Harris, Jensen, Joffe & Mason (1944) found, by biological assay with rats, that D-α-tocopherol was about 50% more active than the DL form, D-β-tocopherol about 100% more active than the DL form and D-γ-tocopherol more active than the DL form; several other examples are given in Table 2. It is likely that different ratios may be found when other types of test are used. There may also be a species difference, since Quaife (1952), using the liver-storage test in the rat, found that the D form of α-tocopherol was about twice as active as the DL form, whereas Pudelkiewicz, Matterson, Potter, Webster & Singsen (1960), using the liver-storage test in chicks, found the D form of α-tocopherol to be 1·34 times as active as the DL form. Results of tissue-storage tests (see Table 2) show that the tocopherols are stored in the tissues examined (liver, fat and hen's eggs) in the relative order of their potencies by gestation–resorption assay. This finding has led some workers to suggest that the widely different potencies of the tocopherols are due chiefly to their selective absorption by the intestine. However, when intestinal absorption is by-passed, as in the liver-slice respiration test in which tocopherol is injected directly into the portal vein, the same order of potency is obtained. This would appear to indicate that intestinal absorption is not alone in determining biological activity. The tissue in which the tocopherol functions may also exhibit a selective action, and the type of response of the tissue may also be important. It has been shown by Edwin, Diplock, Bunyan & Green (1961) and by Green, Diplock, Bunyan & Edwin (1961) that, in the rat and the rabbit, there is a high order of tissue selectivity towards tocopherol uptake. Tissue selectivity is further demonstrated by the striking differences between the in vitro and in vivo erythrocyte tests, particularly for ϵ-tocopherol, η-tocopherol and ζ_1-tocopherol.

SUMMARY

1. The potencies of natural ϵ- and ζ_1-tocopherol and DL-5-methyltocol have been compared with that of DL-α-tocopheryl acetate by gestation–resorption assays with rats and found to be 5, 32 and 10%, respectively.

2. It is suggested that the trivial name tocotrienol be given to 2-methyl-2-(4′,8′,12′-trimethyltrideca-3,7,11-trienyl)-6-chromanol, which is the tri-unsaturated analogue of tocol and the parent compound of ϵ- and ζ-tocopherol.

3. Information from various sources on the relative potencies of the tocopherols and methylated tocotrienols is presented and discussed.

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