THE BIOLOGICAL ACTIVITY OF *trans*-4-CARBOXYVITAMIN-A ACID

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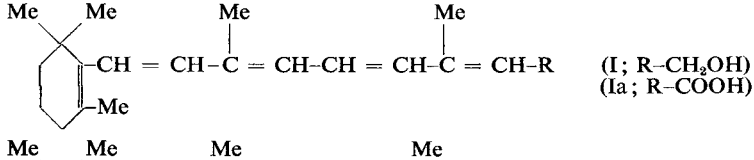
Although the biological activity characteristic of vitamin A (I) is shown by a number of simpler or derived products, the structural features associated with this type of activity have yet to be strictly defined. Marked structural alterations of (I) are known to lead to a pronounced drop in biological potency.[1,2] Replacement of the terminal $-CH_2OH$ group of (I) by carboxyl (Ia), however, gives a compound with biological activity equal to that of the parent vitamin.[3] (Ia)' is, in fact, the most effective compound of this type yet to be synthesised. It was, therefore, of interest to determine the effect, if any, on biological activity exerted by a carboxyl group in position 4. To this end we have synthesised compounds A to D by methods described in detail elsewhere,[4] and now report the biological assay of these compounds employing the growth response of young, vitamin A-depleted, rats as a criterion of potency.

Compound A: *trans*-4-carboxyvitamin-A acid (II).

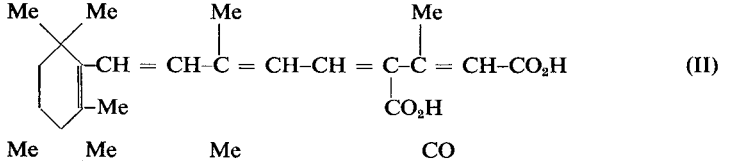
Compound B: Lithium aluminium hydride reduction product of Compound A.

Compound C: *cis*-4-Carboxy-vitamin-A-acid anhydride (III).

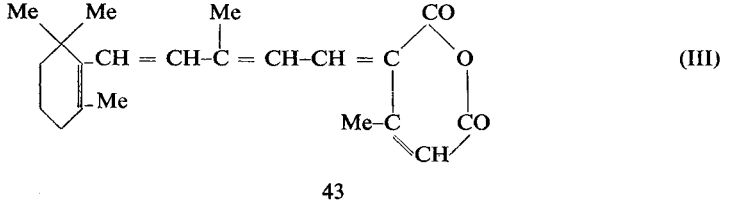
Compound D: The product $C_{22}H_{30}O_7$ obtained by condensing β-ionylidene acetaldehyde with α-carboxy-β-methylglutaconic acid triethyl-ester in the presence of methanolic potash. Two preparations of this compound (D1 and D2) were examined. [The formulation (IV) assigned to compound D from its mode of formation is not supported by light absorption data. Its structure must be considered unproven (cf. ref. 4).]



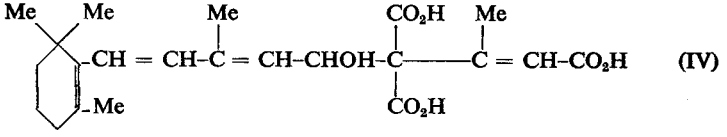
(I; R–CH_2OH)
(Ia; R–COOH)



(II)



(III)



(IV)

EXPERIMENTAL

The vitamin A-deficient diet used had the following composition:—

Fat-free casein	20 per cent.
Rice starch	57 „ „
Salts	5 „ „
Hardened vegetable oil ..	9·9 „ „
Dried yeast	8 „ „
Radiostol solution	0·1 „ „

The rats were placed on the diet at weaning or when they had reached a weight of 40 to 50 g. The animals were of either sex, but litter-mates were always taken in pairs of the same sex. After 3 to 4 weeks on the vitamin A-deficient diet, when depleted of vitamin A, the rats were given the test oils. In each experiment a certain number of animals were kept on the diet with no supplementary dose of vitamin A, as negative controls. In all experiments, groups of animals were given 2 or 3 different doses of vitamin A in the form of either the International standard for vitamin A (β-carotene in 1948-49), or a standardised solution of vitamin A acetate, the potency of which had been previously determined in terms of the International standard.

In experiment No. 3, compound D1, the International standard β-carotene solution, 200 I.U./g., was given in doses of 0·02 g. once or twice

TABLE I

GROWTH RESPONSES

Experiment	Dose per week I.U. or g.		Number of rats	Average growth per week g.
1	Vitamin A acetate (I.U.)	6 3 1·5	10 6 3	3·6 1·8 −1
	Compound A (g.)	0·004	3	1
	Compound C (g.)	0·004	1	−8 (5 died at 3 weeks)
2	Vitamin A acetate (I.U.)	4 2 1	14 12 7	2·7 1·1 0·7
	Compound B (g.)	0·004	21	3·3
3	β-carotene (I.U.)	8 4	5 4	6·8 4·9
	Compound D1 (g.)	0·004	11	4·6
4	Vitamin A acetate (I.U.)	4 2 1	14 10 7	2·7 1·2 0·8
	Compound D2 (g.)	0·004	28	4·1

weekly by pipette, i.e., 4 and 8 units. The animals receiving the dose once a week were given alternate doses of arachis oil.

In the other experiments the standardised solution of vitamin A acetate was diluted 1 : 70 with arachis oil and the potency checked by the Carr-Price blue value. Sub-dilutions were then made for 3 dosage groups: the doses were given in 0·02 g. by pipette. All the compounds were diluted 1 : 10 by weight with arachis oil. Compounds B, D and D2 were first dissolved in ether before the arachis oil was added, the ether then being evaporated off at 37° C. Compound A remained insoluble in ether and so was given as a 1 in 10 suspension in arachis oil. The growth responses are given in Table I. The experimental period was 3 weeks.

The approximate potencies calculated from the figures given in Table I are, therefore, as shown in Table II.

TABLE II

APPROXIMATE POTENCIES

Compound	Estimated potency I.U./g.
A	Approximately 500
B	Approximately 2,000
C	No activity
D1	Approximately 1,000
D2	Approximately 2,000

SUMMARY

The introduction of a carboxyl-group into position 4 of vitamin A and vitamin A acid leads to compounds with only slight biological activity.

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REFERENCES

1. Heilbron, Jones and Richardson, *J. chem. Soc.*, 1949, 287.
2. Heilbron, Jones, Lewis and Weedon, *ibid.*, 2023.
3. Cf. Heilbron, *ibid.*, 1948, 390.
4. Petrow and Stephenson, *ibid.*, 1950, 1311.