

LVII. THE ACTION OF LIGHT ON SUBSTANCES RELATED TO ERGOSTEROL

BY G. A. D. HASLEWOOD

From the British Postgraduate Medical School, London

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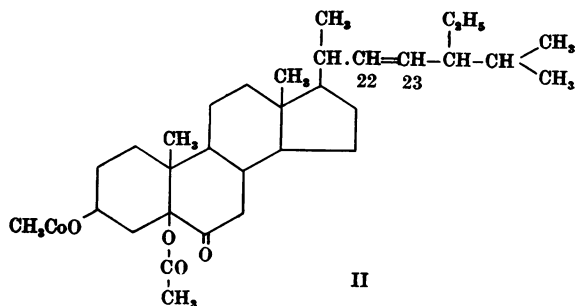
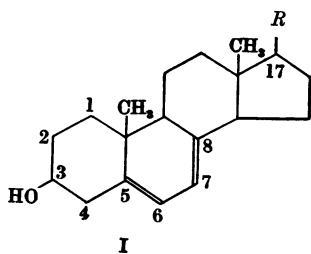
THE brilliant work of Windaus and his colleagues, and of other workers, on 7-dehydrocholesterol [Windaus *et al.* 1935; Windaus *et al.* 1936; Boer *et al.* 1936; Schenck, 1937; Windaus *et al.* 1937] has culminated in the isolation of the related crystalline vitamin D₃, not only from irradiated 7-dehydrocholesterol itself, but also from the liver oil of the tunny [Brockmann, 1936; 1937; Brockmann & Busse, 1937], halibut [Brockmann, 1937], and cod [Zucker *et al.* 1938].

Of particular interest in connexion with this work is the question of the possible existence of other naturally occurring antirachitic substances, in addition to calciferol and vitamin D₃. On biological grounds Bills *et al.* [1937; 1938] consider proven the presence of such compounds in fish liver oils. Further, Windaus & Trautmann [1937] have obtained a crystalline vitamin D₄ from irradiated 22-dihydroergosterol (Ia), while Wunderlich [1936] showed that his 7-dehydrostosterol (I b) possessed provitamin D activity. As Bacharach [1936] emphasized, it seemed remarkable, in view of these results, that the 7-dehydrostigmasterol (I c) of Linsert [1936] had no such properties.

Recently, the "cholanic acid analogue of ergosterol", 3-hydroxy- $\Delta^{5,7}$ -choladienic acid (I d) was prepared, with the use of an improved method for the introduction of the C₇₋₈ double bond [Haslewood, 1938]. This method has now been applied to the preparation of 7-dehydrostigmasterol. The final product was apparently identical with that of Linsert but certain of the intermediates showed different characteristics. In the preparation, also, 3:5-diacetoxy-6-keto- Δ_{22} -sitostene (II) was isolated.

On treatment with sunlight and eosin, in absence of air, the acid (I d) and 7-dehydrostigmasterol both gave crystalline insoluble products, but insufficient material was available for their complete characterization.

- I (a) $R = -\text{CH}(\text{CH}_3) \cdot \overset{22}{\text{CH}_2} \cdot \overset{23}{\text{CH}_2} \cdot \text{CH}(\text{CH}_3) \cdot \text{CH}(\text{CH}_3)_2$.
 I (b) $R = -\text{CH}(\text{CH}_3) \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}(\text{C}_2\text{H}_5) \cdot \text{CH}(\text{CH}_3)_2$.
 I (c) $R = -\text{CH}(\text{CH}_3) \cdot \text{CH}=\text{CH} \cdot \text{CH}(\text{C}_2\text{H}_5) \cdot \text{CH}(\text{CH}_3)_2$.
 I (d) $R = -\text{CH}(\text{CH}_3) \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{COOH}$.
 I (e) $R = -\text{OH}$.



With the generous co-operation of Mr A. L. Bacharach, Dr E. L. Smith and Dr F. A. Robinson of the Glaxo Laboratories, the hydroxycholadienic acid and its ammonium salt, and also 7-dehydrostigmasterol have been irradiated and the products tested on rats for antirachitic activity. The tests, in the Glaxo Laboratories, and in those of the author, showed that the provitamin activity of the acid and its salt was not more than 1/125–1/400 of that of ergosterol; while irradiated 7-dehydrostigmasterol also had not more than 1/125–1/400 of the antirachitic activity of ergosterol similarly treated.

Recently, Dimroth & Paland [1939] have similarly found negative results in the case of the 3:17-dihydroxyandrostadiene (Ie) of Butenandt *et al.* [1938]. Compounds of this group seem to show great specificity in the possession of provitamin properties.

EXPERIMENTAL

Analyses were microanalyses by Dr A. Schoeller. Melting points are uncorrected. Ethereal solutions were dried with anhydrous sodium sulphate.

7-Dehydrostigmasterol. Stigmasterol acetate (10 g., m.p. 142–143°) in acetic acid (500 ml.) was oxidized for a total time of 4 hr. at 50–55° with a solution of chromic anhydride (8 g. CrO₃, in 20 ml. water) and acetic acid (80 ml.); added slowly with continuous stirring during 70 min. After dilution to about 3 l. with water, the product was allowed to stand overnight and the precipitate collected and dissolved in ether. The ether was washed with NaOH and water, dried and evaporated. The residue, from ethyl alcohol, yielded white leaflets of 7-ketostigmasterol acetate, m.p. 184–185° (Linsert: m.p. 183°). The mother liquors were diluted in three stages with water, and filtered twice from crystalline precipitates. Finally, needles were deposited, and these were collected and purified by recrystallization from methyl alcohol and methyl alcohol-light petroleum (twice). In this way, 3:5-diacetoxy-6-keto- Δ_{22} -sitostene (II) was obtained as colourless needles, m.p. 189–190°. (Found: C, 74.8; H, 9.9%. C₃₃H₅₂O₅ requires C, 75.0; H, 9.9%.) 7-Ketostigmasterol acetate (1.5 g.) was reduced with aluminium isopropoxide, purified and the product (1.1 g.) converted into the dibenzoate exactly as described for 7-keto-3-acetoxy- Δ_5 -cholenic acid [Haslewood, 1938]. The dibenzoate of 7-hydroxystigmasterol crystallized from methyl alcohol-acetone in white needles, m.p. 184–186° (Linsert: m.p. 156–158°). (Found: C, 80.9; H, 8.8%. C₄₃H₅₆O₄ requires C, 81.1; H, 8.9%.) This product (0.7 g.) was refluxed for 8 hr. with redistilled dimethylaniline (5 ml.: b.p. 191–192°). The diluted product was acidified with HCl and ether-extracted. Evaporation of the washed and dried ether and crystallization of the residue from ethyl alcohol-benzene yielded white leaflets (0.3 g.). These were refluxed for 15 min. with alcohol (50 ml.), acetone (20 ml.) and 2 N NaOH (2 ml.). The diluted mixture was ether-extracted, and the residue from evaporation of the washed and dried ether was crystallized from alcohol until the product (70 mg.) formed colourless needles of constant m.p. 149–151° (Linsert: m.p. 154°). 7-Dehydrostigmasterol gave the colour reactions of ergosterol. The acetate (prepared in the usual way with acetic anhydride and pyridine at 100° for 15 min.) crystallized from alcohol-benzene in white leaflets, m.p. 169–171° (Linsert: m.p. 172°).

Effect of sunlight and eosin. (a) On 7-dehydrostigmasterol: this substance (15 mg.) with eosin (15 mg.) was gently boiled in a 5 ml. flask with ethyl alcohol (4 ml.) and benzene (1 ml.). While still hot, the flask was tightly stoppered, and was then allowed to stand for some weeks in a sunny place. The precipitated needles were collected, and recrystallized from benzene-alcohol. White crystals m.p. 203–204° were obtained.

(b) On 3-hydroxy- $\Delta^{5,7}$ -choladienic acid: this acid (20 mg.), eosin (20 mg.) and ethyl alcohol (5 ml.), were treated as above in a 5 ml. flask. The product consisted of white needles, M.P. 238–239°.

Tests of provitamin activity. (a) In Glaxo Laboratories: 5 mg. of the compounds tested were irradiated in a quartz tube placed about 4 cm. from a mercury vapour lamp. The solutions were rapidly stirred, and a current of N_2 passed through them during the irradiation. The resulting solutions (except in the case of the ammonium salt of the acid (I), which was fed directly to rats) were evaporated, and the residues dissolved in olive oil and fed to rachitic rats. The technique of the assays was that of the "line" test. The following results were obtained:

Substance tested	Irradiation time (min.)	Activity of product
Hydroxycholadienic acid as the ammonium salt, in water (50 ml.)	2	Not more than 10 I.U. per mg.
The above acid in ethyl alcohol (25 ml.)	2, 5, 15 and 45	Not more than 10 I.U. per mg.
7-dehydrostigmasterol in ethyl alcohol (25 ml.)	3	Not more than 10 I.U. per mg.
Ergosterol in alcohol (25 ml.)	3	Not more than 4000 I.U. per mg.

(b) In the author's laboratory: the mercury vapour lamp was 5.5 cm. from the quartz tube. The residues from evaporation of the solutions were dissolved in olive oil and tested on Wistar rats. The standard (10 I.U.) was an olive oil solution of pure calciferol. The "line" test and X-ray technique were used in the assays. The following results were obtained:

Substance tested	Irradiation time (min.)	Activity of product
Hydroxycholadienic acid (1 mg.) in ethyl alcohol (5 ml.)	10	<25 I.U. per mg.
7-Dehydrostigmasterol (1 mg.) in ether (5 ml.)	10	<25 I.U. per mg.
0.6 mg. of the above in ether (3 ml.)	10	<25 I.U. per mg.

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