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BIOSYNTHESIS OF ECDYSTERONE FROM CHOLESTEROL IN *TAXUS BACCATA*

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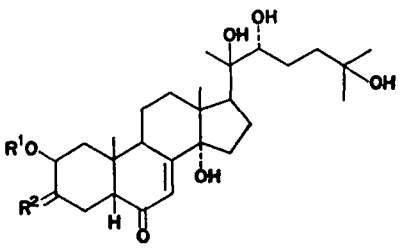
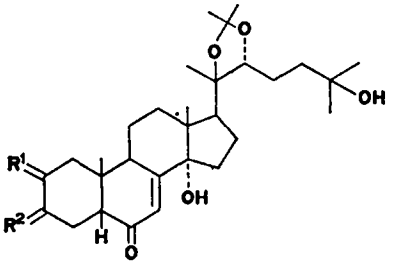
Key Word Index—*Taxus baccata*; Taxaceae; ecdysterone; insect moulting hormone; biosynthesis; cholesterol.

Abstract—Biosynthesis of ecdysterone from [4-^{14}C, 3-^{3}H]cholesterol in *Taxus baccata* does not involve obligatory oxidation at C-3 during the formation of the A/B-*cis* ring junction.

A NUMBER of closely related compounds (ecdysones) possessing insect moulting hormone activity have been isolated from plants;[1] the most common of these is ecdysterone (I). Biosynthetic studies in plants have shown that ecdysterone (I) is derived from mevalonic acid and cholesterol.[2] However, the detailed biosynthetic pathway of ecdysterone from cholesterol, assuming it to be a true *in vivo* precursor, has received little attention.



(I) $R^1 = H$; $R^2 = \beta OH, \alpha H$
(II) $R^1 = Ac$; $R^2 = \beta OH, \alpha H$



(III) $R^1 = \beta OAc$; αH; $R^2 = \beta OH, \alpha H$
(IV) $R^1 = O$; $R^2 = \beta OAc, \alpha H$
(V) $R^1 = \beta OAc, \alpha H$; $R^2 = O$

Saturation of the 5,6-double bond to give a 5β-hydrogen, during the transformation of cholesterol into ecdysterone, can arise by numerous pathways.[1] However, an analogous change during steroid hormone and bile acid[3] formation in animals involves the intermediacy of a 3-keto-Δ^4-steroid. In a similar transformation in plants,[4] radioactive tracer studies have shown oxidation at C-3 to be an obligatory step in the introduction of a 5β-hydrogen into cardenolides biosynthesized from cholesterol in *Digitalis lanata*. In view of these precedents, a 3-keto-Δ^4-steroid could be an intermediate in the biosynthesis of

[1] H. H. REES, in *Aspects of Terpenoid Chemistry and Biochemistry* (edited by T. W. GOODWIN), Chap. 7, Academic Press, London (1971).
[2] N. J. DE SOUZA, E. L. GHISALBERTI, H. H. REES and T. W. GOODWIN, *Phytochem.* 9, 1247 (1970).
[3] R. I. DORFMANN and F. UNGAR, *Metabolism of Steroid Hormones*, Academic Press, New York (1965). H. DANIELSSON and T. T. TCHEN, in *Metabolic Pathways* (edited by D. M. GREENBERG), Vol. 2, p. 118, Academic Press, New York (1968).
[4] E. CASPI and G. M. HORNBY, *Phytochem.* 7, 423 (1968).

moulting hormones in plants. In contrast, Sauer and co-workers[5] reported that [4-^{14}C]-cholest-4-en-3-one was not a precursor of ecdysterone in *Podocarpus elata* seedling.[5] However, it is possible that a 3-keto-Δ^4-steroid could be involved at a later stage, e.g. after insertion of some hydroxyl groups. To obtain more conclusive evidence regarding the possible role of a 3-keto intermediate in moulting hormone biosynthesis, ecdysterone was biosynthesized from [4-^{14} C,3α-^{3}H]cholesterol in *Taxus baccata* seedlings, which are known to convert cholesterol into ecdysterone.[6]

[4-^{14}C, 3-^{3}H]Cholesterol (^{3}H:^{14}C radioactivity ratio 2·77; ^{14}C radioactivity of 20 μCi) was administered to *Taxus baccata* seedlings over a 5-week period, as described in the literature.[5] The seedlings were processed in the usual manner (Table 1) and the resulting butanol extract, after dilution with cold ecdysterone, (5 mg) was chromatographed on silica gel GF_{254} (developed with chloroform–isopropanol, 9:5). The ecdysterone zone (^{14}C radioactivity of 1·1 × 10^4 dpm), after further dilution with cold ecdysterone (20 mg), was crystallized to constant specific radioactivity and ^{3}H:^{14}C radioactivity ratio (Table 2). The isolated ecdysterone had a ^{3}H:^{14}C radioactivity ratio of 3·05 indicating retention of the tritium label at the C-3 position.

TABLE 1. DISTRIBUTION OF RADIOACTIVITY IN EXTRACTS FROM *Taxus baccata* SEEDLINGS ADMINISTERED [4-^{14}C, 3α-^{3}H]CHOLESTEROL (^{14}C RADIOACTIVITY OF 20 μCi; ^{3}H:^{14}C RADIOACTIVITY RATIO 2·77)

Extracts	^{14}C radioactivity	Extracts	^{14}C radioactivity	Extracts	^{14}C radioactivity
Leaf washings	5 μCi	Hexane extract	10 μCi	Butanol extract	3 × 10^5dpm

It is noteworthy that the cholesterol recovered from the plant had a lower ^{3}H:^{14}C radioactivity ratio than the administered cholesterol, viz. 2·66 and 2·77, respectively. In addition, the isolated ecdysterone had a ^{3}H:^{14}C ratio of 3·05 equivalent to a 15% increase in tritium label over the cholesterol recovered from the extracted leaves. The observed changes in ^{3}H:^{14}C radioactivity ratios could be ascribed to the loss of 4% of the tritium label from the absorbed cholesterol in a nonspecific oxidative step. This tritium, could be reintroduced into the biosynthesized ecdysterone either by a similar oxidative reductive mechanism operating on any of the three secondary hydroxyl groups or by transfer to the C-5 position during the formation of the A/B-*cis* ring junction. A similar transfer of label has been observed in the microbial transformation of cholesterol into coprostanol.[7]

If the above conjecture is true, the tritium label within the isolated ecdysterone could be present at positions other than C-3. To establish whether oxidation at C-3 is an obligatory step in the biosynthetic pathway, it was necessary to demonstrate whether the majority of the tritium label within the isolated ecdysterone was located at C-3.

Acetylation of (I) with acetic anhydride in pyridine at 0° for 2 hr, gave, after preparative TLC [chloroform–isopropanol, 3:1], 2β-acetoxyedcysterone (II). Treatment of (II) in acetone with a few drops of 5% perchloric acid, gave after standing at room temp. for 2 hr, 2β-acetoxy-20,22-acetonidecdysterone (III): m.p. 206–210° PMR ($CDCl_3$) δ 0·82 (S, C-13 Me); 1·02 (S, C-10 Me); 1·20 (S, C-20 Me); 1·26 (S, C-25, gem dimethyl), 1·34 and 1·42

[5] H. H. SAUER, R. D. BENNETT and E. HEFTMANN, *Phytochem.* **7**, 2027 (1968).
[6] N. J. DE SOUZA, E. L. GHISALBERTI, H. H. REES and T. W. GOODWIN, unpublished results.
[7] I. BJORKHEM and J. A. GUSTAFSON, *Eur. J. Biochem.* **21**, 428 (1971).

(acetonide methyls): 2·10 (S, AcO); 3·00–3·20 (*m*, C-5H); 3·60–3·74 (*m*, C-22H), 4·08–4·20 (*m*, C-3H, W1/2 4 Hz); 4·88–5·14 (*m*, C-2H, W1/2 10 Hz), 5·86 (*d*, *J* 1·5 Hz, C-7H) ppm *m/e* 562 (very weak M^+); 405 (23%) ($C_{23}H_{33}O_6$) (M^+-157); 387 (42%) (M^+-(157 + 18)); 345 (63%) (M^+-(157 + 60)); 327 (37%) (M^+-(157 + 60 + 18)); 201 (16%) ($C_{11}H_{21}O_3$) (M^+-361); 143 (50%) (201–58); 125 (42%) (143–18); 102 (base peak).

TABLE 2. ^3H:^{14}C RADIOACTIVITY RATIOS OF CHOLESTEROL AND ECDYSTERONE (TOGETHER WITH ITS CHEMICAL TRANSFORMATION PRODUCTS) ISOLATED FROM *Taxus baccata* SEEDLINGS ADMINISTERED [4-^{14}C, 3α-^{3}H]CHOLESTEROL

Compound		Specific radioactivity (dpm/mg)	^3H:^{14}C radioactivity ratio
Cholesterol (administered)	1	201	2·81
	2	220	2·81
	3	228	2·73
Cholesterol (recovered)	1	1457	2·66
	2	1497	2·66
	3	1411	2·66
Ecdysterone (diluted)	1	126	3·04
	2	124	3·05
	3	125	3·06
2β-Acetoxy-20,22-acetonid Ecdysterone	1 2	120 120	3·11 3·21
2β-Acetoxy-20,22-acetonid 3-oxoecdysterone + 25% 2-oxo 3β-acetoxyderivative	—	—	1·5

Oxidation of (III) with Jones reagent,[8] at 0° for 0·5 min, gave a mixture of 3β-acetoxy-20,22-acetonid-2-oxoecdysterone (IV) and 2β-acetoxy-20,22 acetonid-3-oxoecdysterone (V) which were inseparable by TLC. The former compound arises by acyl migration from C-2 to C-3 under the reaction conditions employed. The PMR spectrum of the mixture exhibited peaks at δ 0·84 (*S*, C-14 Me), 1·81 (*S*, 3-20Me), 1·24 (*S*, C-25 *gem*-dimethyl), 134 and 140 (*d*, acetonide methyls), 2·16 (*S*, AcO), 3·58–3·74 (*m*, C-22H), 5·32–5·60 (*m*, Aco CH), 5·90 (*d*, *J* 1·5 Hz, C-7H) ppm. The above signals are consistent with the expected PMR spectrum of either the 3-oxo or 2-oxo steroids. The presence of two C-10 methyl signals at δ 0·87 and 0·79 ppm indicated a mixture of (IV) and (V). The low field signals was assigned to the 3-oxo-steroid (V) as the C-3 carbonyl would be expected to have a larger deshielding influence than the C-2 carbonyl upon the C-10 methyl signal.[9] From the PMR spectrum it was estimated that the mixture contained 25% of the 2-oxo-steroid (IV) and 75% of the 3-oxo-steroid (V). Detailed analysis of the MS of the oxidized product revealed the expected fragmentation pattern, which is consistent with those reported for other 20,22-acetonide derivatives of ecdysterone.[10] *m/e* 560 (very weak) (M^+) ($C_{32}H_{48}O_8$); 467 (14%) (M^+-(60 + 18 + 15)); 403 (22%) ($C_{23}H_{31}O_6$) (M^+-157); 385 (31%) (M^+-(157 + 118)); 343 (22%) (M^+-(157 + 60)); 325 (17%) (M^+-(157 + 60 + 18)); 201 (13%)

[8] C. DJERASSI, R. R. ENGLE and A. BOWERS, *J. Org. Chem.* **21**, 1547 (1956).
[9] N. S. BACCA and D. H. WILLIAMS, *Applications of NMR Spectroscopy in Organic Chemistry*, Holden Day, New York (1964).
[10] M. N. GALBRAITH and D. H. S. HORN, *Austral. J. Chem.* **22**, 1045 (1969).

($C_{11}H_{21}O_3$) (M^+-359): 143 (40%) (201–58); 125 (33%) (201-(58 + 18)); 102 (base peak). The fragments of *m/e* 201, 143 and 125 are assigned to the fragmentation of the side chain and are common fragments in the MS of (III) and (V/IV), confirming that the side chain remains intact during oxidation.

The biosynthesized ecdysterone was transformed as described above and the H^3:^{14}C radioactivity ratio of the products are given in Table 2. Transformation of the radioactive ecdysterone did not result in a drop in the ^3H:^{14}C radioactivity ratio, which confirmed that both the isotopic labels were associated with ecdysterone and not with some extraneous impurity.

Oxidation of the 2β-acetoxy-20,22-acetonidecdysterone (III) gave an oil which had ^3H:^{14}C radioactivity ratio of 1·5. After allowing for the 25% acyl migration from C-2 to C-3 under the reaction conditions, the drop in ^3H:^{14}C radioactivity ratio indicates that 70% of the tritium label present in the biosynthesized ecdysterone was located at C-3.

As discussed earlier, the biosynthesized ecdysterone contains more tritium label than the absorbed cholesterol isolated (estimated as 15% from radioactivity ratios), which is probably the result of an *in vivo* nonspecific dehydrogenase operating on cholesterol and the biosynthesized ecdysterone. An accurate estimation of the degree of oxidation-reduction occurring at C-3 during ecdysterone biosynthesis is not possible. However irrespective of this, which is consistent with the observation by Sauer *et al.*[5] that [^{14}-4C]cholestenone was not transformed into ecdysterone, provides to support the argument that oxidation at C-3 is not an obligatory step in the introduction of an A/B-*cis* ring function in the biosynthesis of ecdysterone by *Taxus baccata*. Further work is in progress to establish the pathway effecting the introduction of a 6-keto group and a 5β-hydrogen into ecdysterone.

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