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SHORT COMMUNICATION

INCORPORATION OF 5α-FUROSTAN-3β,26-DIOL INTO TIGOGENIN BY *DIGITALIS LANATA*

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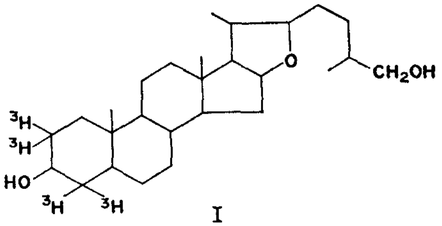
Abstract—2,2',4,4'-$^3H_4$-dihydrotigogenin was converted by *Digitalis lanata* plants into tigogenin.

The biosynthesis of steroidal sapogenins has been widely investigated during the last few years Labelled cholesterol and cholestanol were found to be converted into labelled spirostanols in *Digitalis lanata* However, only few intermediates are known in the biosynthetic route leading from these precursors to sapogenins Bennett *et al* [1] showed the incorporation of 26-^{14}C-26-hydroxycholesterol into diosgenin by *Dioscorea floribunda*, and Tschesche and Fritz[2] reported the incorporation into sapogenins, by *Digitalis lanata*, of 5,6-$^3H_2$-16β,26-dihydroxycholestanol. This last result has been confirmed by us, in simultaneous unpublished research, by incorporation of 2,2',4,4'-$^3H_4$-16β,26-dihydroxycholestanol into tigogenin.

In order to find other possible intermediates we tested the incorporation, by *Digitalis lanata*, of 2,2',4,4'-$^3H_4$-dihydrotigogenin (2,2',4,4'-$^3H_4$-5α-furostan-3β,26-diol) (I) This compound was synthesized by oxidation of 26-*O*-trityldihydrotigogenin to the 3-keto-derivative, removal of the trityl group by acidic hydrolysis, followed by isotopic exchange with CH_3O^3H/NaOH and $NaBH_4$ reduction



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The above compound was administered to *D. lanata* plants simultaneously with 4-^{14}C-cholesterol, which served as an internal control ($^3H/^{14}C$ ratio = 18 5) After 4 weeks the plants were harvested and the labelled tigogenin was isolated and purified as acetate After dilution with carrier 3-acetyltigogenin and crystallization to constant specific activity, the material was hydrolysed and the recovered tigogenin crystallized and counted The presence of tritium in tigogenin from this experiment (see Table 1) indicates that dihydrotigogenin is transformed into tigogenin by *D lanata* plants

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[1] R D Bennett, E Heftmann and R A Joly, *Phytochem* 7, 2021 (1968)
[2] R Tschesche and R Fritz, *Z Naturforsch* B 25, 590 (1970)
[3] R A Joly, J Bonner, R D Bennett and E Heftmann, *Phytochem* 8, 857 (1969)

TABLE 1 RADIOACTIVITY IN TIGOGENIN AND 3-ACETYLTIGOGENIN

Products	Specific activity (dpm of ^{14}C/mM) $\times 10^{-5}$	$^{3}H/^{14}C$ ratio
Tigogenin	3 55	28 9
3-Acetyltigogenin	3 35	27 1

The above results are consistent with the hypothesis that the biosynthesis of tigogenin occurs through the pathway: cholesterol → 26-hydroxycholesterol (or 26-hydroxycholestanol) → 16β,26-dihydroxycholestanol → dihydrotigogenin → tigogenin, that is *via* a furostan This is consistent with the fact that in *Dioscorea floribunda*, cholesterol-4-^{14}C gave a furostanol sapogenin[3] which was converted to diosgenin by the plant[4]

EXPERIMENTAL

2,2′,4,4′-$^{3}H_4$-*Dihydrotigogenin* (I) 3-Acetyldihydrotigogenin (70 mg) was tritylated with $(C_6H_5)_3$ CCl in pyridine at room temp for 24 hr Chromatography of the crude product on Al_2O_3 II yielded, in the fractions eluted with C_6H_6, a mixture of $(C_6H_5)_3$ COH and 3-acetyl-26-*O*-trityl-dihydrotigogenin, which was subjected to hydrolysis with 0 5 N methanolic KOH and rechromatographed on Al_2O_3 II Elution with C_6H_6 yielded $(C_6H_5)_3$ COH, whereas C_6H_6-EtOAc (98 2) eluted 68 mg of 26-*O*-trityldenvative of dihydrotigogenin (ν_{max} in $CHCl_3$ 3530 cm^{-1}), which was oxidized with CrO_3-pyridine to give, after purification by chromatography on Al_2O_3 II (elution with C_6H_6-EtOAc 99 1), 35 mg of 3-keto-26-trityloxyfurostane, m p 107–9°, ν_{max} in $CHCl_3$ 1710 cm^{-1} This last compound, when dissolved in 95% aq HOAc and kept overnight at room temp, furnished 13 mg of 3-keto-26-hydroxyfurostane, m p 121–3°, ν_{max} in $CHCl_3$ 3530, 1710 cm^{-1}, which was tritiated by refluxing for 5 hr its solution in 3 5 ml of 0 1 N NaOH in CH_3O^3H

The tritiated compound (12 mg), recovered in the usual manner, was stirred with $NaBH_4$ (18 mg) in 3 ml of C_2H_5OH for 16 hr Normal work-up yielded 10 mg of 2,2′,4,4′-$^{3}H_4$-dihydrotigogenin, which was purified by repeated TLC The purified compound had a total activity of 4 07 × 10^9 dis/min of ^{3}H

Administration of the radioactive steroids to the plants A mixture of 2,2′,4,4′-$^{3}H_4$-dihydrotigogenin (4 07 × 10^9 dpm) and 4-^{14}C-cholesterol (2 2 × 10^8 dpm, $^{3}H/^{14}C$ ratio = 18 5) was administered to the leaves of *Digitalis lanata* plants in acetone After 4 weeks the plants were harvested and the tigogenin was recovered by known procedures,[5] purified as the acetate by chromatography, diluted with carrier material and crystallized from acetone to constant specific activity Hydrolysis of 3-acetyltigogenin with 5% methanolic KOH yielded tigogenin, which was crystallized from acetone until a constant specific activity was reached

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[4] R A JOLY, J BONNER, R D BENNETT and E HEFTMANN, *Phytochem* **8,** 1445 (1969)

[5] J VON EUW and T REICHSTEIN, *Helv Chim Acta* **47,** 711 (1964)

Key Word Index—Digitalis lanata, Scrophulariaceae, tigogenin, biosynthesis, dihydrotigogenin, sapogenins