Phytochemistry, 1975, Vol. 14, pp. 2423–2425. Pergamon Press. Printed in England.

THE ROLE OF (25*S*)-5α-CHOLESTAN-3β,26-DIOL AND (25*S*)-5α-FUROSTAN-3β,26-DIOL IN THE BIOSYNTHESIS OF TOMATIDINE AND NEOTIGOGENIN

FIAMMA RONCHETTI,* GIOVANNI RUSSO,* GINO FERRARA† and GIUSEPPE VECCHIO†

* Istituto di Chimica Organica della Facoltà di Scienze-Centro per lo studio delle Sostanze Organiche Naturali del C.N.R., Via Saldini 50, 20133 Milan, Italy; and
† Laboratorio per la Chimica degli Ormoni del C.N.R.-Milano

(*Received* 28 *January* 1975)

Key Word Index—*Lycopersicon pimpinellifolium*; Solanaceae; tomato; biosynthesis; tomatidine; neotigogenin.

Abstract—(25*S*)-5α-Cholestan-3β,26-diol was incorporated into neotigogenin and tomatidine, and (25*S*)-5α-furostan-3β,26-diol only in neotigogenin, by *Lycopersicon pimpinellifolium*.

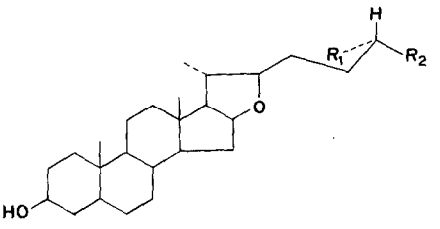
INTRODUCTION

The main steps of the biosynthetic pathway leading from cholesterol [1,2] to (25*R*)-sapogenins have been elucidated [3–6].

The first step of this process may be the hydroxylation at C-26 of cholesterol, whereas the closure to a tetrahydrofuran ring is one of the last steps, as indicated [5] by the incorporation of (25*R*)-5α-furostan-3β,26-diol (**1**).

The details of the biosynthesis of (25*S*)-sapogenins and spirosolanes are less known. An important question is whether their formation occurs through the same sequence of steps leading to (25*R*)-sapogenins. Moreover, it is of interest to ascertain in which stage of the biosynthesis of steroidal alkaloids the introduction of nitrogen occurs.

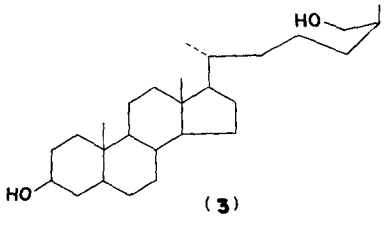
In this connection, we decided to test the incorporation of (25*S*)-5α-cholestan-3β,26-diol (**3**) and of (25*S*)-5α-fucostan-3β,26-diol (**2**) into neotigogenin (**4**), a (25*S*)-sapogenin, and tomatidine (**5**), a (25*S*)-spirosolane, to verify if the hydroxylation



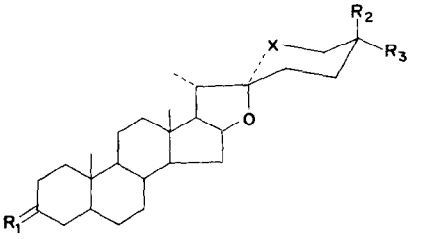
(**1**) $R_1 = CH_2OH$, $R_2 = Me$

(**2**) $R_1 = Me$, $R_2 = CH_2OH$

(**9**) Δ^5; $R_1 = CH_2NH_2$, $R_2 = Me$



(**3**)



(**4**)	$R_1 = \overset{OH}{H}$	X = O	R_2 = Me	R_3 = H
(**5**)	$R_1 = \overset{OH}{H}$	X = NH	R_2 = Me	R_3 = H
(**6**)	$R_1 = \overset{OAc}{H}$	X = O	R_2 = Me	R_3 = H
(**7**)	$R_1 = \overset{OAc}{H}$	X = NAc	R_2 = Me	R_3 = H
(**8**)	R_1 = O	X = O	R_2 = Me	R_3 = H
(**10**)	$R_1 = \overset{OH}{H}$	X = NH	R_2 = H	R_3 = Me; Δ^5

Table 1. Incorporation of precursors into tomatidine and neotigogenin in *Lycopersicon pimpinellifolium*

Precursor	Compound	Radioactivity (dpm × 10^{-3} of ^{14}C/mM)	^{3}H:^{14}C ratio
(25*S*)-5α-cholestan-3β,26-diol-[2,4,2′,4′-$^{3}H_4$]-cholesterol-[4-^{14}C] ($1·1 \times 10^{8}$ dpm of ^{14}C; ^{3}H:^{14}C ratio = 6·5)	Neotigogenin (**4**)	6·25	15·13:1
	Acetylneotigogenin (**6**)	6·43	15·89:1
	Tomatidine (**5**)	308	0·83:1
	N,O-diacetyltomatidine (**7**)	312	0·82:1
(25*S*)-5α-furostan-3β,26-diol-[2,4,2′,4′-$^{3}H_4$]-cholesterol-[4-^{14}C] ($1·1 \times 10^{8}$ dpm of ^{14}C; ^{3}H:^{14}C ratio = 6·0)	Neotigogenin (**4**)	71·5	69·47:1
	Acetylneotigogenin (**6**)	74·1	68·03:1
	Tomatidine (**5**)	116	0·08:1
	N,O-diacetyltomatidine (**7**)	112	0·00:1

at C-26 and the formation of a tetrahydrofuran ring such as (**1**) are general steps occurring also in the biosynthesis of these compounds.

RESULTS AND DISCUSSION

(25*S*)-5α-Cholestan-3β,26-diol-[2,4,2′,4′-$^{3}H_4$] (**3**) was prepared from neotigonenin (**4**) [9] and fed, together with cholesterol-[4-^{14}C], to 3 young plants of *Lycopersicon pimpinellifolium*.

Neotigogenin (**4**) and tomatidine (**5**) were extracted, purified and counted. As shown in Table 1, both the compounds were tritium-labelled.

The results indicate that (25*S*)-5α-cholestan-3β,26-diol (**3**) is incorporated into both neotigogenin (**4**) and tomatidine (**5**), being better incorporated than cholesterol into the former compound.

(25*S*)-5α-Furostan-3β,26-diol-[2,4,2′,4′-$^{3}H_4$] (**2**) was synthesized from tritiated neotigogenone (**8**), mixed with cholesterol-[4-^{14}C] and fed to 3 young plants of *L. pimpinellifolium*. In this experiment, neotigogenin (**4**), but not tomatidine (**5**), was tritium-labelled (Table 1). This observation indicates that (**2**) can act as a precursor of neotigogenin (**4**), but it is not incorporated into tomatidine (**5**).

This result, coupled with the incorporation [7] of 26-aminodihydrodiosgenin (**9**) into solasodine (**10**), suggests that the introduction of the nitrogen function at C-26 must occur before the formation of the tetrahydrofuran ring. This of course would not apply if the (25*R*)- and (25*S*)-spirosolanes are formed through a different sequence of steps but this seems unlikely.

The data obtained are consistent with the hypothesis that the formation of a tetrahydrofuran, such as (**1**) or (**2**) is a common step only in the biosynthesis of (25*R*)- and (25*S*)-sapogenins, suggesting the occurrence of the following pathway:

cholesterol → (25*S*)-5α-cholestan-3β,26-diol → tomatidine
↓
(25*S*)-5α-furostan-3β,26-diol → neotigogenin.

EXPERIMENTAL

Preparation of (25*S*)-5α-*furostan-3β,26-diol*[2,4,2′,4′-$^{3}H_4$] (**2**). Neotigogenone (**8**) was dissolved in 0·1 N NaOH *in iso*-Pr$O^{3}H$ and refluxed under N_2 for 5 hr. After evaporation *in vacuo* at room temp. solid residue was dissolved in $CHCl_3$, washed with H_2O, dried over Na_2SO_4 and evaporated *in vacuo*. Tritiated neotigogenone was reduced with $NaBH_4$ in EtOH to yield neotigogenin-[2,4,2′,4′-$^{3}H_4$], which was separated by preparative TLC (C_6H_6–EtOAc, 9:1) from the 3α-isomer and hydrogenated over PtO_2 in HOAc for 24 hr. After purification by preparative TLC (C_6H_6–EtOAc, 3:2) (**2**) was obtained chromatographically pure.

Administration of radioactive precursors to tomato plants. The labelled precursors were administered, dissolved in Me_2CO, to 3 young plants of *L. pimpinellifolium*, 3 × per week, for 4 weeks. After each administration, plants were sprayed with 10% silicone oil in petrol.

Isolation and purification of neotigogenin (**4**). 1 week after the last administration, the plants were harvested, dried, powdered and extracted [8]. The whole extract was hydrolyzed with 1 N ethanolic HCl and hydrolyzate chromatographed on Si gel–celite: C_6H_6–Et_2O (9:1) eluted crude neotigogenin (**4**), which was purified by preparative TLC (C_6H_6–EtOAc, 19:1) dil. with carrier neotigogenin and acetylated. The acetate (**6**) was purified by preparative TLC (C_6H_6–EtOAc, 19:1) crystallized to const. act. (see Table 1) and finally hydrolyzed to neotigogenin (**4**) which showed, after repeated crystallizations the same sp. act. and ^{3}H:^{14}C ratio as acetate (Table 1).

Isolation and purification of tomatidine (**5**). The more polar fraction of the chromatography of the hydrolyzate, eluted with C_6H_6–Et_2O (3:2) to Et_2O, contained radioactive tomatidine (**5**) which was dil. with carrier material and crystallized to const. sp. act. (Table 1). Acetylation with Ac_2O–Py afforded *N,O*-diacetyltomatidine (**7**), which was purified by preparative TLC (C_6H_6–EtOAc, 7:3) and repeatedly crystallized and counted (Table 1).

Acknowledgements—We thank Prof. Luigi Canonica for helpful discussions and Simes S.p.A. for growing the plants.

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