Phytochemistry, 1980, Vol. 19, pp. 1449–1451. Pergamon Press Ltd. Printed in England.

# SIDE CHAIN FUNCTIONALIZATION OF CHOLESTEROL IN THE BIOSYNTHESIS OF SOLASODINE IN *SOLANUM LACINIATUM**

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(*Received* 28 *August* 1979)

**Key Word Index**—*Solanum laciniatum*; Solanaceae; biosynthesis; steroidal alkaloids; solasodine.

**Abstract**—(25*R*)-26-Amino-cholesterol-[7α-$^3$H], (25*R*)-26-amino-5-cholestene-3β,16β-diol-[7α-$^3$H] and (25*R*)-26-acetylamino-5-cholestene-3β,16β-diol-[7α-$^3$H] administered to *Solanum laciniatum* were converted into solasodine. The results indicate that in the biosynthesis of solasodine the introduction of nitrogen occurs immediately after the hydroxylation at C-26 and before a further oxidation of the side chain of cholesterol. The next step after the amination at C-26 is not hydroxylation at the 16β-position but probably the functionalization of C-22.

## INTRODUCTION

In the biosynthesis of C-27 steroidal alkaloids of the spirosolane type, to which solasodine (**1**), soladulcidine (**2**) and tomatidine (**3**) belong, previous results [1–5] indicate that the functionalization of the cholesterol side chain starts with the introduction of a hydroxy group at C-26. (25*R*)-5α-Cholestane-3β,26-diol (**7**) and (25*S*)-5α-cholestane-3β,26-diol (**13**) were converted stereospecifically into soladulcidine (**2**) [1] and into tomatidine (**3**) [2], respectively, whereas 22-oxo-5α-cholestane-3β-ol [3], 5α-cholestane-3β,16β-diol [4] and 22-oxo-5α-cholestane-3β,16β-diol [5] were not incorporated in spirosolanols.

The C-25 epimeric 5α-cholestane-3β,16β,26-triols (**8** and **14**) were converted only into tigogenin (**4**) [6] and neotigogenin (**5**) (Tschesche, R., Saito, Y. and Töpfer, A., unpublished results), respectively, and not into tomatidine (**3**) ([6]; Tschesche, R., Saito, Y. and Töpfer, A., unpublished results). These results indicate that the introduction of nitrogen could occur after the hydroxylation at C-26. In order to answer the question whether the amination at C-26 takes place immediately after the primary hydroxylation and prior to a further functionalization of the side chain, we synthesized (25*R*)-26-aminocholesterol (**9**) from diosgenin (**6**) [7]. Compound **9** was labelled with tritium at the 7α-position [8] and administered together with cholesterol-[4-$^{14}$C] to *S. laciniatum*. Supposing that the introduction of nitrogen at C-26 is the first step during the formation of the aza-oxa-spiran structure, the question is raised regarding the sequence of further oxidations at C-16 and C-22. In this connection we decided to test the incorporation of (25*R*)-26-amino-5-cholestene-3β,16β-diol (**11**) into solasodine (**1**). Compound **11** was synthesized from diosgenin (**6**) [7], labelled through 7α-bromo-tritium substitution [8], and administered together with cholesterol-[4-$^{14}$C] to *S. laciniatum*.

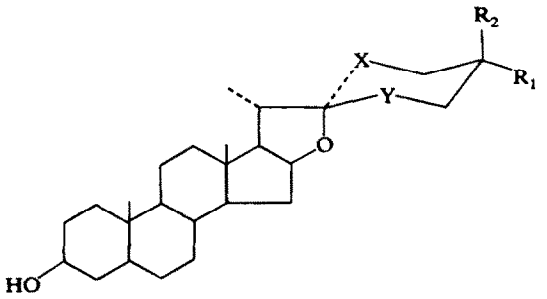
During the experiments we found that **9** and **11** were sensitive to air and therefore we synthesized the 26-acetylamino-cholesterols **10** and **12** [7] which showed no decomposition. Compound **12** was labelled with tritium and administered together with cholesterol-[4-$^{14}$C] to *S. laciniatum* [9].

## RESULTS AND DISCUSSION

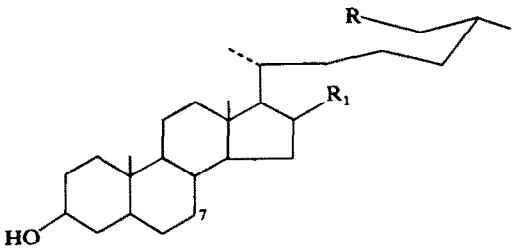
After exposure of the 26-aminocholesterols **9** and **11** to air they decomposed mainly to less polar compounds. The decomposition rates of **9** and **11** were nearly identical. After purification of (25*R*)-26-amino-5-cholestene-3β,16β-diol-[7α-$^3$H] by TLC it was dissolved in $CH_2Cl_2$–MeOH, exposed to air in an open flask for 4 hr and 60 hr, and then chromatographed on Si gel plates ($CHCl_3$–EtOH–$NH_3$ 25%, 85:14:1) and the plates scanned for radioactivity. Very little decomposition was observed after 4 hr but only about 55% of the initial radioactive material remained unchanged after 60 hr and two radioactive peaks were observed near the solvent front.

Similar decomposition processes probably occurred on the leaves of plants after leaf application of **9** and **11**. During the incubation period of 30 days, larger amounts of **9** and **11** decomposed and hence they could not be converted into solasodine (**1**). The loss in radioactivity as a result of this process has not been considered in the calculation of $^3$H-incorporations, since it was unknown. Therefore, the incorporation rates obtained, for **9** and **11** were minimum figures, which had to be corrected in relation to the $^{14}$C-incorporation of cholesterol. A correction factor of

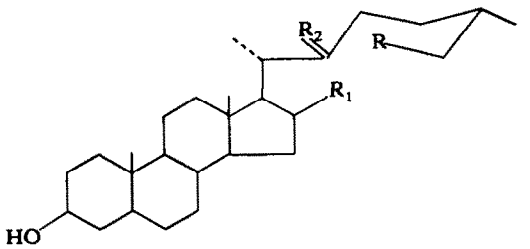
* Part 25 in the series "Biosynthesis of Steroid Derivatives in the Plant Kingdom". For Part 24 see Tschesche, R. and Führer, W. (1979) *Chem. Ber.* **112**, 2692.



**1** $\Delta^5$, X = NH, Y = $CH_2$, $R_1$ = Me, $R_2$ = H (solasodine)
**2** 5$\alpha$H, X = NH, Y = $CH_2$, $R_1$ = Me, $R_2$ = H (soladulcidine)
**3** 5$\alpha$H, X = $CH_2$, Y = NH, $R_1$ = Me, $R_2$ = H (tomatidine)
**4** 5$\alpha$H, X = O, Y = $CH_2$, $R_1$ = Me, $R_2$ = H (tigogenin)
**5** 5$\alpha$H, X = O, Y = $CH_2$, $R_1$ = H, $R_2$ = Me (neotigogenin)
**6** $\Delta^5$, X = O, Y = $CH_2$, $R_1$ = Me, $R_2$ = H (diosgenin)



**7** 5$\alpha$H, R = OH, $R_1$ = H
**8** 5$\alpha$H, R = OH, $R_1$ = OH
**9** $\Delta^5$, R = $NH_2$, $R_1$ = H
**10** $\Delta^5$, R = NHAc, $R_1$ = H
**11** $\Delta^5$, R = $NH_2$, $R_1$ = OH
**12** $\Delta^5$, R = NHAc, $R_1$ = OH



**13** 5$\alpha$H, R = OH, $R_1$ = H, $R_2$ = $H_2$
**14** 5$\alpha$H, R = OH, $R_1$ = OH, $R_2$ = $H_2$
**15** $\Delta^5$, R = OH, $R_1$ = H, $R_2$ = O
**16** $\Delta^5$, R = OH, $R_1$ = H, $R_2$ = 

72.6 was established by comparing the $^3H$ : $^{14}C$ ratios of **11** and **12**. In spite of imponderable circumstances, such as different rates of absorption, transport, etc., the apparently better conversion of **12** mainly resulted from the instability of **11**.

The administered compounds were converted into solasodine (**1**) to different extents by *S. laciniatum*. The relative incorporations and the ratio of the $^3H$- and $^{14}C$-incorporations are shown in Table 1.

(25*R*)-26-Aminocholesterol (**9**) was converted into solasodine (0.43%). After applying the correction factor 72.6 to the $^3H$ : $^{14}C$ ratio it increased from 0.56 to 40.8. The real ratio which could be achieved if **9** were a stable compound must be between 0.56 and 40.8, and certainly it should be greater than one. Hence, there is every reason to believe that (25*R*)-26-aminocholesterol (**9**) can be converted into solasodine (**1**) more readily than can cholesterol. Thus, the biogenetic introduction of nitrogen can occur immediately after the hydroxylation at C-26.

In a previous experiment [1] the existence of a C-26 oxointermediate stage was not observed. This observation, coupled with the incorporation of **9** into solasodine, indicates that a direct substitution of the 26-hydroxy group, which could be activated by phosphorylation, for a $NH_2$ or NHR group must occur. Whether the introduction of nitrogen is possible after the oxidation at C-22 remains to be tested. However, Kaneko *et al.* [10] surmised that this occurred in the biosynthesis of 22,26-epiminocholestenes after the isolation of dormantione (**15**) and dormantinol (**16**) from budding *Veratrum* sp.

The small incorporation of (25*R*)-26-amino-5-cholestene-3$\beta$,16$\beta$-diol (**11**) (0.0095%), the unfavourable $^3H$ : $^{14}C$ ratio (0.0027) compared to **9**, and the inferior incorporation of (25*R*)-26-acetylamino-5-cholestene-3-$\beta$,16$\beta$-diol (**12**) in relation to cholesterol (0.196) indicates that the hydroxylation at C-16 does not occur immediately after the amination of C-26. This verifies our recent results [11] that the hydroxyl group at C-16 is introduced only after the formation of ring F. As compound **11** was not converted into solasodine on the main biosynthetic pathway, we think that after the amination of C-26 the functionalization of C-22 will occur and ring F will probably be formed during the next step.

Table 1. Incorporation of the administered compounds into solasodine by *S. laciniatum*

| Compounds | Specific activity × $10^{-5}$ (dpm/mmol) | Incorporation (%) | $^3H$ : $^{14}C$ ratio |
|---|---|---|---|
| (25*R*)-26-Amino-cholesterol-[7$\alpha$-$^3$H], | 421.31 | 0.431 | 0.562 (min) |
| Cholesterol-[4-$^{14}$C] | 28.67 | 0.767 | (corr. 40.8) |
| (25*R*)-26-Amino-5-cholestene-3$\beta$,16$\beta$-diol-[7$\alpha$-$^3$H], | 0.39 | 0.0095 | 0.0027 (min) |
| Cholesterol-[4-$^{14}$C] | 7.61 | 3.48 | (corr. 0.196) |
| (25*R*)-26-Acetylamino-5-cholestene-3$\beta$,16$\beta$-diol-[7$\alpha$-$^3$H], | 17.46 | 0.172 | 0.196 |
| Cholesterol-[4-$^{14}$C] | 4.79 | 0.874 | |

## EXPERIMENTAL

*Methods.* Radioactivity measurements were made with a liquid scintillation counter. Quench corrections were made using the external standard ratio method. Aliquots of radioactive samples were dissolved in 15 ml scintillator solution of toluene containing 15 g PPO (2,5-diphenyloxazole) and 187.5 mg POPOP (1,4-bis-[5-phenyloxazol-2-yl] benzene) per 2.5 l. TLC plates were scanned on a Berthold Scanner, model LB 2723.

*Preparation of labelled compounds.* (25*R*)-26-Aminocholesterol (**9**) and (25*R*)-26-amino-5-cholestene-3β,16β-diol (**11**) were prepared from diosgenin (**6**) via (25*R*)-26-phthalimidocholesterol and via (25*R*)-26-phthalimido-5-cholestene-3β,16β-diol, respectively, as described previously [7]. After acetylation and allylic bromination with *N*-bromosuccinimide the 7α-bromo-cholesterols were reduced catalytically with tritium [8] (Radiochemical Centre, Amersham). Hydrazinolysis, followed by saponification in methanolic KOH yielded [7α-$^3$H]-**9** and [7α-$^3$H]-**11**. [7α-$^3$H]-**12** was prepared by esterfication of [7α-$^3$H]-**11** with $Ac_2O$ in MeOH [7]. The labelled compounds were purified by PTL on 1 mm layers of Si gel [$CHCl_3$–EtOH–$NH_3$ 25%, 93:7:1 (**9**), $CHCR_3$–EtOH–$NH_3$ 25%, 85:14:1 (**11**), and $CHCl_3$–EtOH, 93:7 (**12**)]. Cholesterol-[4-$^{14}$C] (sp. act. 57 mCi/mmol) was obtained from the Radiochemical Centre, Amersham.

*Administration of labelled compounds.* After mixing with cholesterol-[4-$^{14}$C] each of the labelled compounds was administered, dissolved in EtOH, to *ca* 40 leaves of 6 *S. laciniatum* plants. The wax layers were removed from these leaves before the administration with 1% TWEEN 20 in water. After the administration the treated leaves were sprayed with silicone oil–petrol (1 : 1).

*Isolation and purification of solasodine* (**1**). About 30 days after the administration, the plants were harvested, extracted and worked up as previously described [1]. After chromatography on a Si gel column the crude solasodine was purified by PLC (2× with $CHCl_3$–MeOH, 15 : 1 and then $CHCl_3$ saturated with $NH_3$ gas), diluted with carrier solasodine and repeatedly crystallized to constant sp. act. (Table 1) in different solvent mixtures (MeOH,$CH_2Cl_2$,EtOH,$CHCl_3$).

*Acknowledgements*—The authors thank Prof. Dr. H. Kating, Institut für Pharmazeutische Biologie der Universität Bonn, for permission for use of the greenhouse and Mr. Echtle for growing the plants. Financial support by the Deutsche Forschungsgemeinschaft and the Friedrich-Ebert-Stiftung is gratefully acknowledged.

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