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# BIOSYNTHESIS OF CHOLESTEROL FROM POLLINASTANOL IN THE TOBACCO *NICOTIANA TABACUM*

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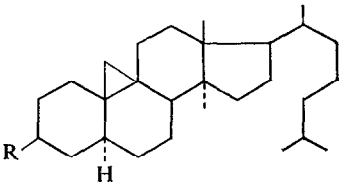
**Abstract**—Pollinastanol (I), tritiated in position 3, is incorporated into cholesterol by leaves of the tobacco *Nicotiana tabacum* Wisconsin with a 1% yield. As expected, however, $C_{28}$ and $C_{29}$ sterols were unlabelled. The significance of these results is discussed.

## INTRODUCTION

POLLINASTANOL (I), a new 14-methylsterol, was first found in a mixed pollen of unknown composition[1,2] and after a systematic search, was isolated from the pollens of *Hypochoeris radicata* and *Taraxacum dens-leonis*.[3] The stereochemistry of I at rings C/D junction was not established, but because of its presumed biogenetic relationship to cycloartenol and cholesterol, the configuration shown in I was suggested. Cycloartenol, but not lanosterol, has also been found in different pollens.[4] (For further information concerning the biosynthesis of phytosterols from cycloartenol, see Refs. 5–8.)




(I) R = OH Pollinastanol, m.p. 111–112°.
(II) R = $CH_3COO$— Pollinastanyl acetate, m.p. 86–90° $[\alpha]_D^{20°} = +25° \pm 2°$ ($HCCl_3$).

In the course of *in vivo* experiments with possible phytosterol precursors, we recently observed the incorporation of tritiated cyloartanol into cholesterol, in the tobacco *Nicotiana tabacum*.[9] In the present paper, we report the incorporation of 3-$^3$H-pollinastanol into the cholesterol by tobacco leaves.

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## RESULTS AND DISCUSSION

Pollinastanol (I) was incorporated into leaves of *Nicotiana tabacum* with a yield of *ca.* 1 per cent. Preparative GLC showed that all the incorporated radioactivity was present in cholesterol (using two different sterol derivatives on two different stationary phases). It was shown that all the labelling was still in position 3 by obtaining the non-radioactive 3-ketones of the sterol fraction through the Oppenauer oxidation. Lack of randomization was also established by the fact that no radioactivity was present either in a sample of the distilled fatty acids or in stigmasterol.

Using the same techniques of isolation, we failed to demonstrate the presence of noticeable amounts of pollinastanol as a constituent of the tobacco leaves. In preceding experiments[9] on the incorporation of tritiated cycloartanol into cholesterol in the same plant, we were also unable to find any radioactivity in an authentic pollinastanol introduced as a carrier. If pollinastanol is present in tobacco leaves, therefore, it must occur in very small amounts indeed.

It does not seem possible on the results so far obtained to decide whether pollinastanol is really a precursor of cholesterol or if it is an intermediate between cycloartenol and cholesterol as suggested earlier.[4] However, its transformation into cholesterol in reasonable yield indicates that it might be an intermediate, for example, in the pollens where it has been found.

The incorporation of pollinastanol into cholesterol does suggest that the proposed stereochemistry (I) for pollinastanol is correct, especially concerning the rings C/D junction.

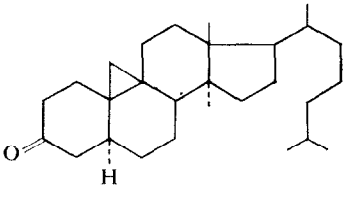
## METHODS AND MATERIALS

*Purification of Pollinastanol*

Pollinastanol (I) (from pollen of *Taraxacum dens leonis*) was isolated as its acetate (II) by preparative TLC on $AgNO_3$/silicic acid,[3] m.p. 86–90°* (crystallized from MeOH); $[\alpha]_D^{20°} = +25 \pm 2°$ ($CHCl_3$). In order to eliminate traces of unsaturated sterols, further TLC was carried out after epoxidation with *p*-nitroperbenzoic acid.[10,11] Saponification of the recovered acetate (II) led to the free pollinastanol (I), m.p. 111–112° (crystallized from MeOH). The purity of this sample was further checked by GLC of the trimethylsilyl ether,[12] NMR and mass spectrometry.[1,2,13]

3-$^3$H-*Pollinastanol*

*Pollinastanone.* The 3-ketone (III) was prepared by the usual Oppenauer oxidation.[14] The crude pollinastanone was purified by TLC on silicic acid using hexane–ethyl acetate, 9:1, as solvent and 2,4-dinitrophenylhydrazine HCl-ide for detection ($R_f$ 0·48). After one crystallization from MeOH, the pollinastanone (III) melted at 98·5–99°.




(III) Pollinastanone, m.p. 98·5–99°.

* All melting points were measured with the Kofler apparatus and are corrected.

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3-$^3$H-*Pollinastanol.* 4 mg of III, dissolved in 1·5 ml of tetrahydrofuran, were treated for 30 min with 1 mc of tritiated $NaBH_4$; 10 mg of $NaBH_4$ were added and, after 30 min, the reaction mixture was dried under vacuum. The residue was taken into $Et_2O$ and filtered through a small column of silicic acid in order to obtain a clear solution. The tritiated pollinastanol was submitted to a preparative TLC on silicic acid (hexane–ethyl acetate, 9:1); a second purification was effected on $AgNO_3$/alumina* with the solvent system light petroleum–$CHCl_3$–acetone, 7:12:1, $R_f$=0·67. 2 mg of 3-$^3$H-pollinastanol were obtained, with a total activity of $10^9$ c/m. The acetate (acetic anhydride/pyridine at 37°) was isolated by preparative TLC on $AgNO_3$-impregnated alumina (hexane–ethyle acetate, 87:3, $R_f$=0·75), and the pure compound (1 mg) had a total radioactivity of $3{\cdot}2\times10^8$ c/m. Further purification by repetition of the preparative TLC on a small part of this sample showed no decrease in radioactivity.

*Incorporation*

The tritiated pollinastanyl acetate, in ether ($4\times10^7$ c/m), was sprayed on leaves of two young plants of *Nicotiana tabacum.* After 5 days, the leaves were extracted as described previously.[9,15,16] The acetone-soluble lipids (2 g) had a total radioactivity of $3\times10^7$ c/m (approximate counts due to quenching, the raw products being highly coloured) and all the radioactivity was found in the unsaponifiable fraction (0·5 g).

*Isolation of Sterols*

Sterols were isolated by column chromatography of the unsaponifiable fraction on silicic acid (Mallinckrodt), the elutions being followed by the Liebermann–Burchard reaction and by TLC. The total sterols, still containing unused precursor, had a radioactivity of $2{\cdot}2\times10^7$ c/m. They were separated from the precursor by preparative TLC on $AgNO_3$-impregnated alumina (light petroleum–$CHCl_3$–acetone, 7:12:1, $R_f$=0·33); 15 mg were recovered of total radioactivity $4{\cdot}2\times10^5$ c/m. This last procedure was repeated three times, but at the second TLC the radioactivity was constant ($3{\cdot}3\times10^5$ c/m). The propionates were prepared from this material[16–18] and separated in two groups by preparative TLC on $AgNO_3$/alumina using the solvent system

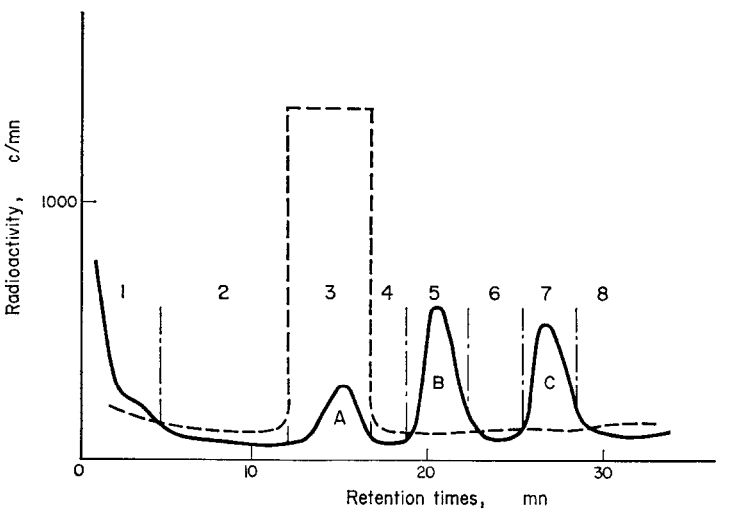


FIG. 1. GLC OF THE STEROL PROPIONATES CONTAINING THE CHOLESTERYL PROPIONATE.

——— Detector signal. – – – – Radioactivity (measurements on the fractions recovered at the end of the column). The figures from 1 to 8 indicate the fractions collected. A: cholesteryl propionate; B: campesteryl propionate; C: β-sitosteryl propionate.

* Important differences have been noticed in the fractionation of sterol derivatives through TLC on $AgNO_3$-impregnated alumina due to the quality of alumina. Merck neutral type T alumina has been generally used in this work. Fluka, type DO, 2 μ, and Bio-Rad neutral AG7, 2–44 μ, aluminas have also been tried with success in some cases.

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light petrol–ethyl acetate, 190:3·5. The fraction of $R_f$=0·55 contained the propionates of campesterol, β-sistosterol and cholesterol as verified by GLC and mass spectrometry; (for an analysis of the sterols of *N. tabacum* Wisconsin, see Ref. 16. The total radioactivity of this fraction was $8 \times 10^4$ c/m. A second fraction ($R_f$=0·40) contained the stigmasteryl propionate with a radioactivity of $2 \times 10^2$ c/m.

*Location of the Radioactivity in Cholesterol*

The sterol fraction containing the propionates of campesterol, β-sitosterol and cholesterol was injected in a gas chromatograph using a silicon gum SE 30 (1%)-celite HMDS column and a temperature of 230° The presence of three compounds was observed, having the same retention times as the authentic propionates (Fig. 1). Eight different fractions were collected at the end of the column and their radioactivity measured separately. All the radioactivity was present in the cholesteryl propionate. This analysis was repeated with the trimethylsilyl derivatives of the sterols and a QF-I 10%-celite HMDS column; all the radioactivity was found in the trimethylsilyl ether of cholesterol.

The propionates of campesterol, β-sitosterol and cholesterol were transformed into the 3-ketones by Oppenauer oxidation[14] (on the free sterols obtained through saponification). TLC on silicic acid (pentane–ethyl acetate, 7:3) showed a single spot with 2,4-dinitrophenylhydrazine/HCl ($R_f$=0·60). None of these 3-ketones were radioactive, as expected from the initial labelling of the sterols in position 3. This result also indicates lack of randomization. The possibility of randomization was furthermore checked by control of the radioactivity on a sample of distilled fatty acids (mainly consisting of palmitic acid), and by preparing from the isolated stigmasteryl propionate (see above) a diepoxide propionate of stigmasterol which was not radioactive (after purification by TLC).

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