Phytochemistry, 1967, Vol. 6, pp. 837 to 840. Pergamon Press Ltd. Printed in England

SHORT COMMUNICATION

BIOSYNTHESIS OF NEOTIGOGENIN AND Δ^{16}-5α-PREGNEN-3β-OL-20-ONE FROM CHOLESTEROL IN *LYCOPERSICON PIMPINELLIFOLIUM*

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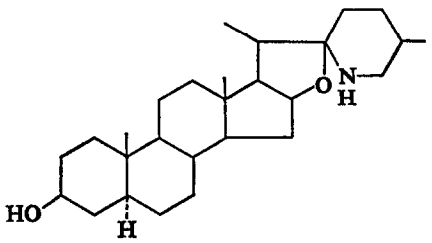
(*Received* 14 *December* 1966)

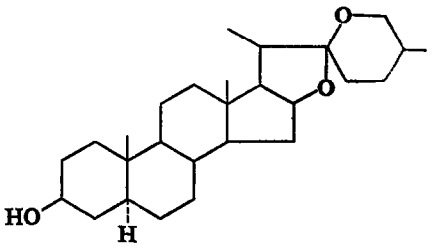
Abstract—Radioactive neotigogenin and Δ^{16}-5α-pregnen-3β-ol-20-one were isolated from the flowers of *Lycopersicon pimpinellifolium* plants which had been treated with cholesterol-4-^{14}C. Both were purified to constant specific activity by chromatography and recrystallization of the acetates as well as the free sterols. Since radioactive tomatidine had previously been isolated from the same plants, the simultaneous biosynthesis of the structurally analogous steroidal sapogenins and alkaloids from cholesterol has now been demonstrated.

INTRODUCTION

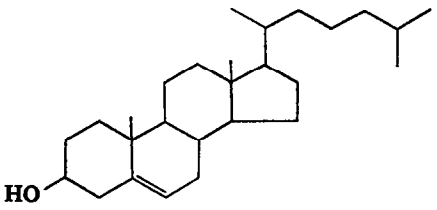
THE presence of both the steroidal alkaloid tomatidine (I)[1] and the structurally analogous sapogenin neotigogenin (II)[2] in *Lycopersicon pimpinellifolium* makes this tomato plant useful for biosynthetic studies. Previously, we observed that cholesterol-4-^{14}C (III) is converted by



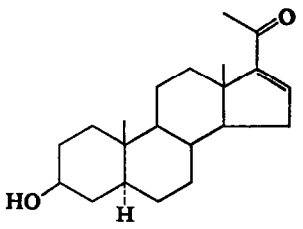
(I) Tomatidine



(II) Neotigogenin



(III) Cholesterol



(IV) Δ^{16}-5α-Pregnenolone

* A laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture. Work conducted under a co-operative agreement with the California Institute of Technology.

[1] T. D. FONTAINE, G. W. IRVING, JR., R. MA, J. B. POOLE and S. P. DOOLITTLE, *Arch. Biochem.* **18**, 467 (1948).
[2] K. SCHREIBER, *Abhandl. Deut. Akad. Wiss. Berlin, Kl. Chem. Geol. Biol.* 143 (1956).

L. pimpinellifolium to tomatidine,[3] which is the nitrogen analog of neotigogenin, and by *Dioscorea spiculiflora* to the sapogenin diosgenin,[4] which differs from neotigogenin in having a double bond at C-5 and the opposite configuration at C-25. The present work demonstrates that, as expected, cholesterol is also a precursor of neotigogenin in *L. pimpinellifolium.*

Recently Schreiber and Aurich reported that *L. pimpinellifolium* contains Δ^{16}-5α-pregnen-3β-ol-20-one (IV),[5] which can also be obtained by chemical degradation of tomatidine.[6] We have now found that this compound is also made from cholesterol in *L. pimpinellifolium.*

RESULTS

In the previous paper[3] we described the isolation of radioactive tomatidine from an extract of *Lycopersicon pimpinellifolium* plants, which had been treated with cholesterol-4-^{14}C, by chromatography on an alumina column. Two of the earlier fractions from this column appeared to contain radioactive neotigogenin and Δ^{16}-5α-pregenenolone. These fractions were combined and the two radioactive steroids were isolated by preparative thin-layer chromatography (TLC). The neotigogenin was further purified by preparative TLC in a different system and acetylated, and neotigogenin acetate was obtained in chromatographically pure form by TLC. This material was then diluted with authentic neotigogenin acetate and shown to be radiochemically pure by crystallization from two solvents, conversion to neotigogenin, and two more crystallizations (Table 1). The same sequence of methods was used to purify the Δ^{16}-5α-pregnenolone and to demonstrate its radiochemical homogeneity (Table 2).

TABLE 1. RECRYSTALLIZATION OF NEOTIGOGENIN ACETATE AND NEOTIGOGENIN*

Compound	Solvent used for crystallization	Counts/min/μmole†
Neotigogenin acetate		18·9±0·9
	Methanol	18·7±0·9
	Hexane	18·9±0·9
Neotigogenin	Hexane–dichloromethane	18·0±0·9
	Acetone	18·1±0·9

* 0·2-mg portions were plated from chloroform solutions on ringed planchets over an area of 12·7 cm^2 and counted in duplicate on a Beckman Widebeta II instrument. Counter efficiency was 34 per cent and background was 1·5 counts/min.

† 90 per cent confidence level.

TABLE 2. RECRYSTALLIZATION OF Δ^{16}-5α-PREGNENOLONE ACETATE AND Δ^{16}-5α-PREGNENOLONE*

Compound	Solvent used for crystallization	Counts/min/μmole
Δ^{16}-5α-Pregnenolone acetate		34·8±1·8
	Hexane	27·7±1·4
	Hexane	27·1±1·4
	Methanol	27·5±1·4
Δ^{16}-5α-Pregnenolone	Hexane–acetone	26·5±1·4
	Hexane–dichloromethane	26·9±1·4

* Conditions as in Table 1.

[3] E. HEFTMANN, E. R. LIEBER and R. D. BENNETT, *Phytochem.* **6**, 225 (1967).
[4] R. D. BENNETT and E. HEFTMANN, *Phytochem.* **4**, 577 (1965).
[5] K. SCHREIBER and O. AURICH, *Phytochem.* **5**, 707 (1966).
[6] Y. SATO, A. KATZ and E. MOSETTIG, *J. Am. Chem. Soc.* **73**, 880 (1951).

DISCUSSION

The biosynthesis of sapogenins from cholesterol has previously been demonstrated in *Dioscorea spiculiflora*[4] and *Digitalis lanata*.[7] Thus, *Lycopersicon pimpinellifolium* is the third plant in which this transformation has been observed, which suggests that cholesterol may be a general precursor of sapogenins. It now appears that the biosynthetic pathways of tomatidine and neotigogenin coincide at least as far as cholesterol.

The biosynthesis of Δ^{16}-5α-pregnenolone from cholesterol demonstrated here is consistent with the findings that the latter is a precursor of the isomeric Δ^{5}-pregnen-3β-ol-20-one in *Haplopappus heterophyllus*[8] and *Digitalis purpurea*,[9] as well as of all pregnane derivatives in animals.[10]

Because only the flowers of the plants were worked up,[3] it was not possible to determine the per cent conversion of cholesterol to the two steroids in this experiment. Neither is a comparison of their radioactivities to that of tomatidine[3] meaningful. The conditions used for hydrolysis of the glycosides (1 N HCl, 100°, 30 min), while optimal for tomatine, give low yields in the case of saponins.[11, 12]

Sander has shown that tomatine is degraded by tomato fruits as they ripen,[13] and Schreiber and Aurich[5] have suggested that Δ^{16}-5α-pregnenolone may be a product of this degradation. However, when we treated a green tomato fruit with tomatidine-4-^{14}C (prepared by biosynthesis[3]), we found no radioactive Δ^{16}-5α-pregnenolone after the fruit had ripened, although all of the tomatidine had been metabolized. The major products, all more polar than tomatidine and Δ^{16}-5α-pregnenolone, were a neutral material and at least three basic compounds.

EXPERIMENTAL

Thin-layer chromatographic techniques were as described previously.[8] All chromatograms were run on Silica Gel G plates purchased from Analtech, Inc., Wilmington, Delaware.* Aliquots of radioactive samples were counted on planchets at infinite thinness under a gas-flow detector (see Table 1, legend, for details).

Fractions 3 and 4 from the alumina column described in the previous paper[3] were combined (4·3 mg, $2{\cdot}15 \times 10^4$ counts/min). TLC of an aliquot of this material showed the presence of substances corresponding in color and fluorescence (revealed by 50% sulfuric acid[14]), as well as mobility, to cochromatographed standards of neotigogenin and Δ^{16}-5α-pregnenolone. Both of these zones were radioactive and were isolated by preparative TLC in the same system. The neotigogenin zone ($4{\cdot}1 \times 10^3$ counts/min) was further purified by preparative TLC with dichloromethane–ether (99:1, continuous development[15] for 4 hr). The isolated material (1200 counts/min), which was estimated by TLC to contain about 50 μg of neotigogenin, was then acetylated with pyridine–acetic anhydride (1:1). The product was subjected to TLC by continuous development[16] with dichloromethane for 2 hr. The plate was scanned for radioactivity and the neotigogenin acetate zone (revealed by spraying with Rhodamine 6G), which coincided with the major radioactive peak, was removed and eluted (650 counts/min). This material was diluted with pure neotigogenin acetate and crystallized as shown in Table 1. It was then converted to neotigogenin by treatment with lithium aluminum hydride[17] and further crystallized.

* Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

[7] R. Tschesche and H. Hulpke, *Z. Naturforsch.* **21b**, 494 (1966).
[8] R. D. Bennett and E. Heftmann, *Phytochem.* **5**, 747 (1966).
[9] E. Caspi, D. O. Lewis, D. M. Piatak, K. V. Thimann and A. Winter, *Experientia* **22**, 506 (1966).
[10] E. Heftmann and E. Mosettig, *Biochemistry of Steroids*. Reinhold, New York (1960).
[11] E. S. Rothman, M. E. Wall and H. A. Walens, *J. Am. Chem. Soc.* **74**, 5791 (1952).
[12] T. Tsukamoto, T. Kawasaki and T. Yamauchi, *Chem. Pharm. Bull. (Tokyo)* **4**, 35 (1956).
[13] H. Sander, *Planta* **47**, 374 (1956).
[14] E. Heftmann, S.-T. Ko and R. D. Bennett, *J. Chromatog.* **21**, 490 (1966).
[15] R. D. Bennett and E. Heftmann, *J. Chromatog.* **12**, 245 (1963).
[16] R. D. Bennett and E. Heftmann, *J. Chromatog.* **21**, 488 (1966).
[17] R. D. Bennett, E. Heftmann, W. H. Preston, Jr. and J. R. Haun, *Arch. Biochem. Biophys.* **103**, 74 (1963).

The Δ^{16}-5α-pregnenolone zone from above ($6{\cdot}0 \times 10^3$ counts/min) was estimated by TLC to contain about 200 μg of this steroid. TLC of an aliquot of the material with dichloromethane–methanol (97:3) indicated. that it was radiochemically homogeneous. However, after acetylation as above, a radioactive impurity less polar than Δ^{16}-5α-pregnenolone acetate was detected by TLC with cyclohexane–ethyl acetate (17:3). This was removed by preparative TLC in the same system, giving chromatographically pure Δ^{16}-5α-pregnenolone acetate ($1{\cdot}9 \times 10^3$ counts/min), which was diluted with authentic material and crystallized as shown in Table 2. It was then hydrolyzed to Δ^{16}-5α-pregnenolone with sodium hydroxide in methanol[8] and further crystallized.