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BIOSYNTHESIS OF *STROPHANTHUS* CARDENOLIDES FROM PROGESTERONE

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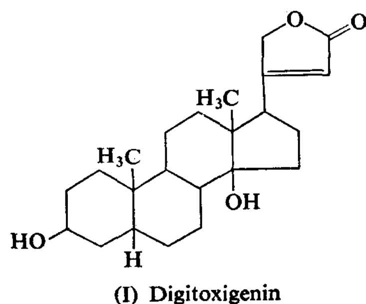
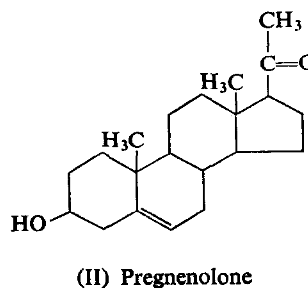
Abstract—After the administration of progesterone-4-^{14}C to *Strophanthus kombé* plants, the cardenolides periplogenin, strophanthidin, and strophanthidol were isolated and found to be radioactive.

INTRODUCTION

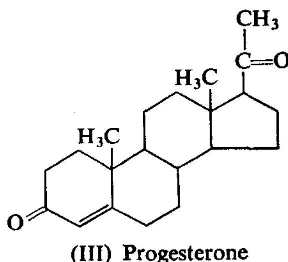
RECENT work on the biosynthesis of *Digitalis* cardenolides, e.g. digitoxigenin (I), has shown that they are made from pregnenolone (II)[1-3] via progesterone (III).[2-4] We have now undertaken a study of the biosynthesis of *Strophanthus* cardenolides, which differ from those of *Digitalis* in being oxygenated at the 5 and 19 positions. Our first objective was to determine if the earlier steps in the biosynthetic pathways are the same in the two species.



(I) Digitoxigenin



(II) Pregnenolone



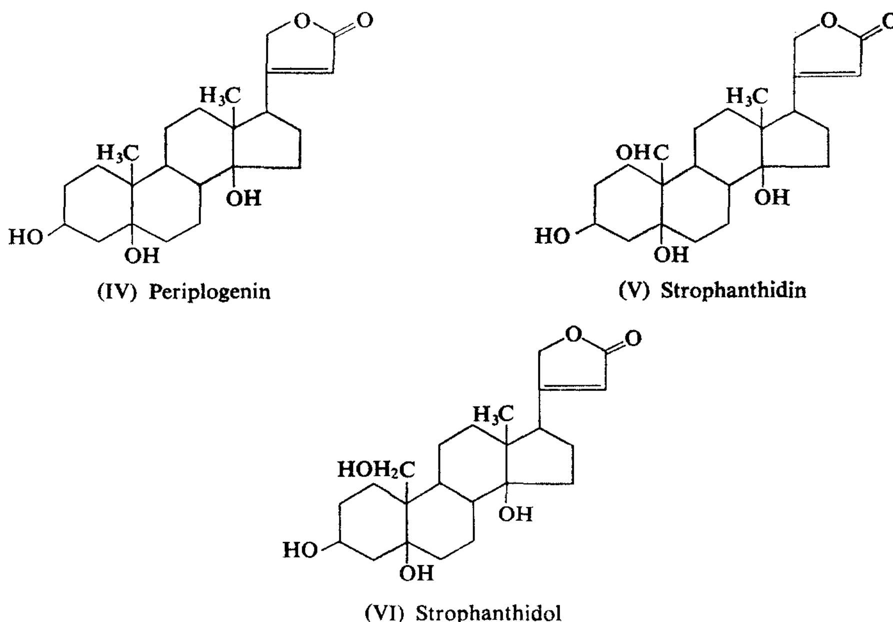
(III) Progesterone

* A laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture. Work conducted under a cooperative agreement with the California Institute of Technology. Requests for reprints should be addressed to E. H.

[1] R. TSCHESCHE and G. LILIENWEISS, *Z. Naturf.* **19b**, 265 (1964).
[2] E. CASPI and D. O. LEWIS, *Science* **156**, 519 (1967).
[3] H. H. SAUER, R. D. BENNETT and E. HEFTMANN, *Phytochem.* **6**, 1521 (1967).
[4] R. D. BENNETT, H. H. SAUER and E. HEFTMANN, *Phytochem.* **7**, 41 (1968).

RESULTS

Progesterone-4-^{14}C was administered once a week for 5 weeks to the leaves of four *Strophanthus kombé* plants. Workup by the usual methods gave, after mild acid hydrolysis, an extract containing the three cardenolides, periplogenin (IV), strophanthidin (V), and strophanthidol (VI). These compounds were separated by column chromatography on



(IV) Periplogenin

(V) Strophanthidin

(VI) Strophanthidol

silica gel and further purified by preparative thin-layer chromatography (TLC). Each of the cardenolides was shown to be radioactive by TLC in three solvent systems. In addition, the strophanthidin was recrystallized from three solvents, and the specific activity remained constant (Table 1).

TABLE 1. RECRYSTALLIZATION OF STROPHANTHIDIN*

Solvent used for crystallization	Counts/min/μmol†
Ether-light petroleum	15,700 ± 800
Methanol-ether-light petroleum	15,100 ± 800
Ethyl acetate	14,700 ± 700

* Portions of 0·2 mg or less were plated from solution 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 2 counts/min.

† 90 per cent confidence level.

DISCUSSION

In animals the transformation of Δ^5-steroids to the saturated 5α- or 5β-analogs is known to proceed via a Δ^4-3-ketone. Therefore, it was not surprising when progesterone was found to be an intermediate in the biosynthesis of the *Digitalis* cardenolides from pregnenolone. The *Strophanthus* cardenolides are 5β-hydroxy-steroids, however, and nothing is known about the biosynthesis of such compounds, since they do not occur in animals. The present findings indicate that a Δ^4-3-ketone, progesterone in this case, may also be an obligatory intermediate of 3,5-diols. Thus, the biosynthesis of both the *Digitalis* and the *Strophanthus* cardenolides apparently proceeds through the same intermediates until the double bond is saturated. In the case of *Strophanthus*, this is followed by hydroxylation at C-5. Work now in progress supports this hypothesis, but an alternative mechanism, direct hydration of the double bond of progesterone, cannot be excluded at this time.

A consideration of the structures of the three *Strophanthus* cardenolides, which differ only in the 19-position, suggests[5] a biosynthetic relationship between them, e.g. periplogenin (IV) → strophanthidol (VI) → strophanthidin (V). This subject is now being investigated. It is also of interest that, in animals, oxygenation at the 19-position is a necessary prerequisite for aromatization of ring A to form the estrogens, which occur in plants as well as animals.[6]

EXPERIMENTAL

Methods

Thin-layer chromatographic techniques were as described previously.[7] 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). Melting points were taken on a Kofler block and are corrected.

Materials

Progesterone-4-^{14}C, having a specific activity of 57·3 $\mu c/\mu M$, was purchased from New England Nuclear Corporation. *Strophanthus kombé* plants were raised from seeds.

Administration of Progesterone

Four *S. kombé* plants, about 1·5 months old and 15 cm long, were each treated with 10 μc of progesterone-4-^{14}C by the technique previously described.[8] A total of five such treatments were given, at weekly intervals. The plants grew to a length of about 40 cm during this period.

Extraction and Fractionation

Three days after the final treatment, the plants were harvested. The portions above the soil line were combined, frozen in liquid N_2, and lyophilized. The dried material (16 g) was homogenized in a blender with 200 ml of 50 per cent ethanol. The homogenate was filtered, and the filter cake was extracted by heating for 30 min at 60° with 200-ml portions of 60, 70, 80, 90, and 100 per cent ethanol in succession. Each extract was filtered, and the combined filtrates were evaporated to a volume of about 100 ml. This concentrate was refluxed with 100 ml of 0·2 N H_2SO_4 and 200 ml of methanol for 30 min. Then 200 ml of water was added, and the methanol was removed in vacuum at 50°. The concentrate was heated at 60° for 30 min and then extracted with three 200-ml portions of hexane. The extracts were washed with 20 ml of water, two 30-ml portions of 0·1 N NaOH, and three 30-ml portions of water, combined and evaporated.

The concentrate was then extracted with three 200-ml portions of dichloromethane. The extracts were washed as above, combined, dried over sodium sulfate, and evaporated.

[5] E. Heftmann, *Ann. Rev. Plant Physiol.* **14**, 225 (1963).
[6] E. Heftmann, *Am. Perfumer Cosmetics* **82**, 47 (1967).
[7] R. D. Bennett and E. Heftmann, *Phytochem.* **5**, 747 (1966).
[8] R. D. Bennett and E. Heftmann, *Phytochem.* **4**, 475 (1965).

* 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.

The concentrate was finally extracted with three 200-ml portions of dichloromethane-ethanol (2:1). The extracts were washed, dried, and evaporated as above.

Hexane extract: 850 mg, $7 \cdot 2 \times 10^7$ counts/min.

Dichloromethane extract: 210 mg, $2 \cdot 6 \times 10^7$ counts/min.

Dichloromethane-ethanol extract: 268 mg, $3 \cdot 2 \times 10^7$ counts/min.

TLC showed that the dichloromethane extract contained the cardenolides. This extract was chromatographed on a 150-g column of silica gel (particle size 0·05–0·2 mm),* packed into a chromatographic tube of 30 mm diameter as a slurry in chloroform. Fractions of about 20 ml each were collected every 30 min with the following eluents: 1–100, 6 per cent; 101–150, 8 per cent; 151–202, 12 per cent; and 203–322, 16 per cent isopropyl alcohol in $CHCl_3$. The fractions were monitored by TLC with $CHCl_3$-isopropyl alcohol (9:1).

Fractions 186–202 (12 mg, $9 \cdot 5 \times 10^5$ counts/min) appeared to contain periplogenin, along with some slightly less polar radioactive material. The periplogenin was isolated by preparative TLC with $CHCl_3$-isopropyl alcohol (9:1), giving 4 mg, $2 \cdot 83 \times 10^5$ counts/min. In two other TLC systems, ethyl acetate and benzene-95 per cent ethanol (4:1), this material corresponded in color and fluorescence after spraying with 50 per cent H_2SO_4 and heating, as well as in mobility, to cochromatographed standards of periplogenin. When scanned for radioactivity, the plates showed a single peak corresponding to periplogenin.

Strophanthidin was found largely in fractions 221–243 (50 mg, $1 \cdot 42 \times 10^6$ counts/min). It corresponded in every respect to cochromatographed standards of strophanthidin when subjected to TLC with $CHCl_3$-isopropyl alcohol (9:1), ethyl acetate-pyridine-water (5:1:4, upper phase) and benzene-95 per cent ethanol (4:1). The strophanthidin was recrystallized to constant specific activity from the solvents shown in Table 1.

Fractions 271–300 (15 mg, $1 \cdot 62 \times 10^6$ counts/min) contained the major part of the strophanthidol, but it was accompanied by a slightly more polar radioactive material which was difficult to separate. Accordingly, the earlier fractions 262–270 (6 mg, $5 \cdot 90 \times 10^5$ counts/min), which contained almost pure strophanthidol, were used for isolation of this cardenolide. Preparative TLC with dichloromethane-methanol (23:2) gave 5 mg, $3 \cdot 80 \times 10^5$ counts/min. TLC with benzene-95 per cent ethanol (4:1) and ethyl acetate-pyridine-water (5:1:4, upper phase) showed that the radioactivity was associated with strophanthidol.

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* Brinkmann Instruments, Westbury, New York.