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CONVERSION OF 5α-PREGNANOLONE TO UZARIGENIN BY *STROPHANTHUS KOMBÉ*

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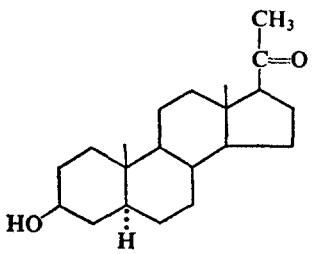
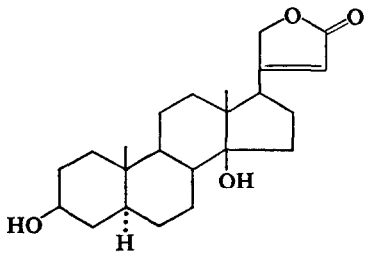
Abstract—Radioactive uzarigenin (II) was isolated from a *Strophanthus kombé* plant which had been treated with 5α-pregnane-3β-ol-20-one-4-^{14}C (I). This 5α-cardenolide was not previously known to occur in that species.

INTRODUCTION

WE PREVIOUSLY found that both *Digitalis lanata* and *Strophanthus kombé* plants convert progesterone-4-^{14}C to 5α-pregnanolone (I), as well as to several cardenolides.[1,2] It now seems likely that in cardenolide biosynthesis the double bond of progesterone is reduced at an early stage.[3–5] Therefore, 5α-pregnanolone appeared to be a plausible biosynthetic intermediate. The high specific activity of this compound in both plants also suggested to us that it is turned over rapidly and may have a precursor role. On the other hand, all the cardenolides known to occur in both *D. lanata* and *S. kombé* have the 5β-configuration, and we would presume that they are made from a 5β-pregnane. We were, therefore, interested to determine the metabolic role of 5α-pregnanolone-4-^{14}C by readministration to a *S. kombé* plant.



(I) 5α-Pregnanolone



(II) Uzarigenin

* 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. D. BENNETT, H. H. SAUER and E. HEFTMANN, *Phytochem.* **7**, 41 (1968).
[2] H. H. SAUER, R. D. BENNETT and E. HEFTMANN, *Phytochem.*, in press.
[3] R. TSCHESCHE, H. HULPKE and H. SCHOLTEN, *Z. Naturforsch.* **22b**, 677 (1967).
[4] E. CASPI and G. M. HORNBY, *Phytochem.* **7**, 423 (1968).
[5] E. CASPI, J. A. F. WICKRAMASINGHE and D. O. LEWIS, *Biochem. J.* **108**, 499 (1968).

RESULTS

A *Strophanthus kombé* plant was treated over a period of 4 weeks by our foliar application method[6] with 5α-pregnanolone-4-^{14}C previously biosynthesized from progesterone-4-^{14}C.[2] The plant was worked up by the previously described procedure[7] to give a hexane extract, containing nonpolar material, and a dichloromethane extract, containing mainly the cardenolides. The hexane extract contained 5α-pregnanolone as the only significant radioactive component. In the dichloromethane extract, however, several radioactive metabolites were observed by TLC. One of these, which corresponded chromatographically to uzarigenin (II), was isolated by column chromatography and preparative TLC. It was then diluted with carrier uzarigenin, crystallized to constant specific activity, acetylated, and again crystallized (Table 1). The incorporation of 5α-pregnanolone-4-^{14}C into uzarigenin was 0·081 per cent.

TABLE 1. RECRYSTALLIZATION OF UZARIGENIN AND UZARIGENIN ACETATE*

Compound	Solvent used for crystallization	Counts/min/μmole†
Uzarigenin		20·8 ± 1·0
	Ether–methanol	18·1 ± 1·0
	Ether–methanol	17·1 ± 0·9
	Ether–methanol	17·0 ± 0·8
	Methanol	16·9 ± 0·8
Uzarigenin acetate		17·2 ± 0·9
	Dichloromethane–ether	17·0 ± 1·2
	Methanol	17·0 ± 1·2

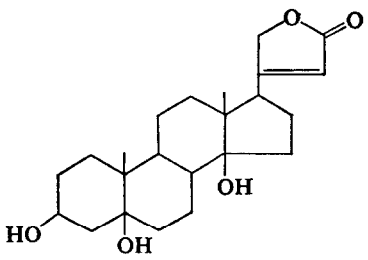
* Portions of 0·2 mg or less were plated from solution on ringed planchets over an area of 12·7 cm^2 and counted in duplicate on a Beckman Widebeta II instrument. Counter efficiency was 34 per cent and background was 2 counts/min.

† 90 per cent confidence level.

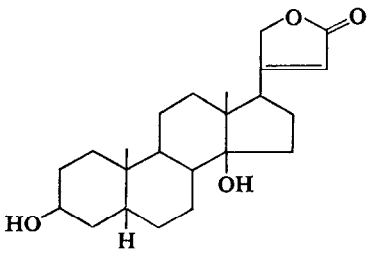
DISCUSSION

Although several metabolic products of 5α-pregnanolone were observed, none of them appeared to be present in weighable quantities. The only one which could be identified was the 5α-cardenolide uzarigenin, which had not previously been detected in *Strophanthus kombé* and is known to occur only infrequently[8,9] in *Strophanthus* species. Tschesche and Snatzke[10] had found 5α-pregnanolone together with uzarigenin in *Xysmalobium undulatum* and suggested a biogenetic relationship between the two, but this had not been demonstrated experimentally until now. The yield of 0·08 per cent compares favorably with the 0·36 per cent incorporation of progesterone-4-^{14}C into periplogenin (III),[2] when it is considered that the latter is present in much higher concentration in *S. kombé* than is uzarigenin.

[6] R. D. BENNETT and E. HEFTMANN, *Phytochem.* **4**, 475 (1965).
[7] H. H. SAUER, R. D. BENNETT and E. HEFTMANN, *Phytochem.* **7**, 1535 (1968).
[8] H. LICHTI, C. TAMM and T. REICHSTEIN, *Helv. Chim. Acta* **39**, 1933 (1956).
[9] J. H. RUSSELL, O. SCHINDLER and T. REICHSTEIN, *Helv. Chim. Acta* **44**, 1315 (1961).
[10] R. TSCHESCHE and G. SNATZKE, *Ann. Chem.* **636**, 105 (1960).



(III) Periplogenin



(IV) Digitoxigenin

The transformation of progesterone to cardenolides involves, in general, introduction of oxygen at the 14- and 21-positions and reduction at the 5-position. The first evidence regarding the sequence in which these changes occur was provided by Tschesche *et al.*,[3] who found that tritiated 5β-pregnane-3β,14β-diol-20-one was converted into digitoxigenin (IV) by *Digitalis lanata*. Caspi *et al.*,[5] on the other hand, demonstrated the existence of the biosynthetic pathway progesterone → 21-hydroxyprogesterone → digitoxigenin in *D. lanata*. These data seem to indicate that more than one pathway of digitoxigenin biosynthesis is operative in this plant. Our present findings show that in *S. kombé* at least one route to uzarigenin starts with reduction of the double bond of progesterone.

The results of our work on *S. kombé* demonstrate the utility of tracer experiments in detecting minor constituents. Previously, only 5β-steroids had been isolated from this plant, but we have now shown that it also contains at least three 5α-compounds. It seems likely that many plant steroids have escaped detection because of their low concentrations.

We have also readministered two 5β-metabolites[2] of progesterone-4-^{14}C, 5β-pregnanolone and 5β-hydroxypregnanolone, to *S. kombé* plants. In both cases there was some indication of incorporation into periplogenin, but the low radioactivity precluded purification to constant specific activity. Likewise, readministration of periplogenin-^{14}C failed to give positive evidence of its conversion into the two 19-oxygenated cardenolides, strophanthidin and strophanthidol.

EXPERIMENTAL

Methods

TLC techniques were as described previously.[2,11] Aliquots of radioactive samples were counted on planchets at infinite thinness under a gas-flow detector (see Table 1, legend, for details).

Administration of 5α-Pregnanolone

5α-Pregnanolone-4-^{14}C was isolated from Fraction 6 of the silica gel column, as described in the preceding paper.[2] The purified material ($1{\cdot}70 \times 10^6$ counts/min), which was radiochromatographically homogeneous by TLC in four systems, was administered in eight equal doses, twice weekly for 4 weeks, to the leaves of a *Strophanthus kombé* plant, 1·5 months old, by the technique previously described.[6]

Isolation of Uzarigenin

The whole plant was frozen in liquid N_2 and lyophilized 5 days after the final treatment. The dried material (9 g) was worked up as previously described,[7] except that extraction with CH_2Cl_2–EtOH was omitted.

The hexane extract (22 mg, $7{\cdot}48 \times 10^5$ counts/min) was examined by TLC with CH_2Cl_2–MeOH (97:3). The major peak in the radioscan corresponded in mobility to 5α-pregnanolone, and a very small peak was observed near the solvent front, probably an ester.[2] TLC of the CH_2Cl_2 extract (15 mg, $8{\cdot}00 \times 10^4$ counts/min) with CH_2Cl_2–MeOH (23:2) showed about nine radioactive peaks of approximately equal intensity. One of

[11] R. D. Bennett and E. Heftmann, *Phytochem.* **5**, 747 (1966).

these corresponded in mobility to 5α-pregnanolone and a second to uzarigenin, but none of the others was identified.

Nine-tenths of the CH_2Cl_2 extract was chromatographed on an 8-g column of silica gel (particle size 0·05–0·2 mm),* packed into a chromatographic tube of 20 mm dia. as a slurry in $CHCl_3$. Fractions of 25 ml each were collected with the following eluents: 1–2, $CHCl_3$; 3–4, 2 per cent; 5–8, 4 per cent; 9–12, 6 per cent; 13–14, 8 per cent; and 15–18, 10 per cent MeOH in $CHCl_3$. TLC with ethyl acetate showed that Fractions 6 and 7 ($2·9 \times 10^4$ counts/min) contained the uzarigenin. These two fractions were combined with 1 mg of carrier uzarigenin and subjected to preparative TLC in the same system. The uzarigenin zone ($4·0 \times 10^3$ counts/min) was then further purified by preparative TLC with CH_2Cl_2–MeOH (9:1), giving $1·6 \times 10^3$ counts/min. This material (1·4 mg) was diluted with 15 mg of carrier uzarigenin, crystallized, acetylated, and again crystallized as shown in Table 1.

Acknowledgements—The authors gratefully acknowledge the assistance of Miss Ellen R. Lieber and Mrs. Barbara Winter.

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