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SHORT COMMUNICATION

METABOLISM OF PROGESTERONE BY *DIOSCOREA DELTOIDEA* SUSPENSION CULTURES

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Abstract—*Dioscorea deltoidea* plant tissue suspension cultures are capable of metabolizing progesterone to 5α-pregnan-3-β-ol-20-one and 5α-pregnan-3β,20β-diol. The latter product has not previously been reported as a metabolic product of progesterone by plant systems. Both transformation products are present as conjugates in this plant tissue culture.

INTRODUCTION

THE MICROBIAL transformations of steroids have been extensively investigated.[1,2] However, there are few reports on the transformation of steroids by plant tissue cultures. Graves and Smith[3] observed that various plant suspension cultures could metabolize pregnenolone to 4-pregnen-20α-ol-3-one and 5α-pregnan-3,20-dione, while progesterone was readily metabolized to 5α-pregnan-3,20-dione and 5α-pregnan-3β-ol-20-one by a variety of cultures. 4-Pregnen-20α-ol-3-one and 4-pregnen-20β-ol-3-one were obtained from progesterone by incubation with *Rosa* tissue cultures.[3] Suspension cultures of *Nicotiana* and *Sophora* have recently been reported to metabolize progesterone and pregnenolone into 5α-pregnan-3β-ol-20-one palmitate.[4] Microsomes from *Dioscorea* and *Cheiranthus* suspension cultures will convert progesterone into 5α-pregnan-3,20-dione.[5] The metabolism of digitoxigenin[6] and cholesterol[7] by plant suspension cultures has also been reported. We here report the biotransformation of progesterone (I) by *Dioscorea deltoidea* suspension cultures to 5α-pregnan-3β-ol-20-one (II), and a new metabolite, 5α-pregnan-3β,20β-diol (III).

RESULTS

Progesterone-4-^{14}C was incubated with *Dioscorea deltoidea* tissue suspension cultures for 30 days. The medium, dried tissue, and acid hydrolyzed tissue were extracted with $CHCl_3$. The distribution of the total extractable radioactivity in these 3 fractions is given in Table 1. Approximately 90% of the $CHCl_3$ extractable radioactivity was present in the acid hydrolyzed tissue extract, the fraction containing steroids that had been present in a conjugated form in the tissues.

[1] W. CHARNEY and H. L. HERZOG, *Microbial Transformation of Steroids*, Academic Press, New York (1967).
[2] H. IIZUKI and A. NAITO, *Microbial Transformation of Steroids and Alkaloids*, University of Tokyo Press and University Park Press, Tokyo (1967).
[3] J. M. H. GRAVES and W. K. SMITH, *Nature, Lond.* **214**, 1248 (1967).
[4] T. FURUYA, M. HIROTANI and K. KAWAQUCHI, *Phytochem.* **10**, 1013 (1971).
[5] S. J. STOHS, *Phytochem.* **8**, 1215 (1969).
[6] S. J. STOHS and E. J. STABA, *J. Pharm. Sci.* **54**, 56 (1965).
[7] S. J. STOHS, B. KAUL and E. J. STABA, *Phytochem.* **8**, 1679 (1969).

TABLE 1. DISTRIBUTION OF ^{14}C AFTER INCUBATING 4-^{14}C-PROGESTERONE WITH *Dioscorea deltoidea* SUSPENSION CULTURES FOR 30 days

Fraction	% Extracted radioactivity
Medium	0·01
Pre-hydrolyzed tissue	0·94
Acid hydrolyzed tissue	99·05

Following the incubation of *D. deltoidea* suspension cultures with 4-^{14}C-progesterone, the medium, tissue, and acid hydrolyzed tissue were extracted with $CHCl_3$. The per cent of total extractable radioactivity in each fraction is given in the table.

Column chromatography of the acid hydrolyzed tissue extract on silica gel yielded two major radioactive peaks upon elution with heptane–ethyl acetate (5:2). These two metabolites co-chromatographed with 5α-pregnan-3β,20β-diol and 5α-pregnan-3β-ol-20-one using TLC in three solvents (see Experimental). The identity of these two products was verified by co-crystallization to constant specific activity with the authentic non-radioactive materials from several solvents (Table 2).

TABLE 2. CO-CRYSTALLIZED 4-^{14}C-PROGESTERONE METABOLITES FROM *Dioscorea deltoidea* TISSUE SUSPENSION CULTURES

Metabolite	Solvent for recrystallization	Specific activity (cpm/mg)
5α-Pregnan-3β-ol-20-one	Hexane–acetone (1:1)	351 ± 39
	Methanol–water (9:1)	339 ± 25
	Ethyl acetate	342 ± 10
5α-Pregnan-3β,20β-diol	Hexane-acetone (1:1)	2250 ± 32
	Methanol–water (9:1)	2390 ± 151
	Ethyl acetate	2510 ± 12
	Ethanol (95%)	2480 ± 40

The two major metabolites of progesterone were isolated by column chromatography. Each was shown to be radiochemically pure by TLC. The corresponding non-radioactive authentic compound was added, followed by re-crystallization to constant specific activity.

The total amount of radioactivity from 4-^{14}C-progesterone present as 5α-pregnan-3β-ol-20-one and 5α-pregnan-3β,20β-diol was determined by co-chromatography and liquid scintillation counting. The diol contained approximately 27% of the ^{14}C present in the conjugate fraction while almost 19% of the radioactivity was present in the 5α-pregnan-3β-ol-20-one (Table 3). The remaining radioactivity was present in a number of small unidentified metabolites.

DISCUSSION

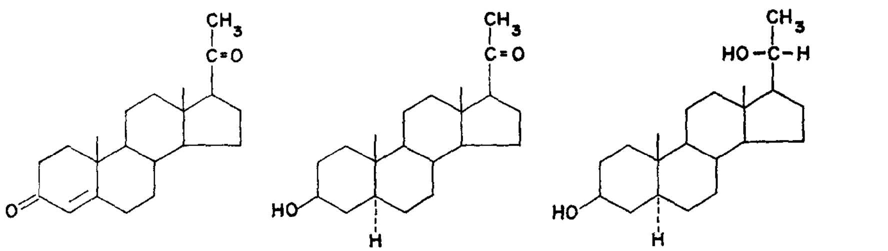
5α-Pregnan-3β,20β-diol has not been previously reported as a major metabolite of progesterone, although 5α-pregnan-3β-ol-20-one has been observed as a product of proges-

TABLE 3. METABOLITES OF PROGESTERONE IN *Dioscorea deltoidea* SUSPENSION CULTURES PRODUCED IN 30 days

Metabolite	% Radioactivity in glycoside fraction
5α-Pregnan-3β-ol-20-one	18·9 ± 1·1
5α-Pregnan-3β,20β-diol	27·2 ± 2·6

The % radioactivity present in each metabolite in the $CHCl_3$ extract of the acid hydrolyzed tissue was determined by co-chromatography on TLC plates, followed by ^{14}C liquid scintillation counting of the silica gel areas corresponding to the metabolites.

terone metabolism by tissue cultures[3,4] and intact plants.[8] The function of the 20β-hydroxysteroid dehydrogenase activity in *Dioscorea* and other plant systems is not known. No significant amount of radioactivity from 4-^{14}C-progesterone could be found associated with diosgenin, other sapogenins, or the sitosterol (sterol) fraction following column chromatography.



(I) Progesterone (II) 5α-Pregnan-3β-ol-20-one (III) 5α-Pregnan-3β,20β-diol

Virtually all of the extractable radioactivity was present in the conjugate fraction, extractable from the dried *Dioscorea* tissue only after acid hydrolysis. These results differ from those of Furuya *et al.*[4] who found that large amounts of progesterone had been converted to 5α-pregnan-3β-ol-20-one palmitate which was present in the neutral $CHCl_3$ tissue extract. These investigations employed *Nicotiana* and *Sophora* tissue cultures, and the authors did not examine the fraction derivable after acid hydrolysis of the tissues.[4]

The presence of most of the radioactivity from progesterone in the conjugate fraction of *D. deltoidea* tissue cultures is consistent with observations on the metabolism of cholesterol[7] and 4-androsten-3,17-dione[9] by these cultures. In metabolic studies using the latter substrate, the presence of 17β-hydroxysteroid dehydrogenase activity in *Dioscorea* tissue cultures has been observed.[9]

EXPERIMENTAL

Plant tissue cultures and progesterone administration. Undifferentiated (callus) suspension cultures of *Dioscorea deltoidea* were grown and maintained as previously described in modified Skoog and Murishiege's medium containing 0·1 ppm 2,4-dichlorophenoxyacetic acid.[7,10] Erlenmeyer incubation flasks (500 ml) contained 100 ml of medium to which was added 25 ml of 10-day-old *D. deltoidea* tissue inoculum and 10 mg progesterone in 0·50 ml 70% EtOH. To 9 of the 27 flasks employed was also added 1·0 μCi 4-^{14}C-progesterone

[8] R. D. BENNETT, H. H. SAUER and E. HEFTMANN, *Phytochem.* 7, 41 (1968).
[9] S. J. STOHS and M. M. EL-OLEMY, *Lloydia* (in press).

(114 μCi/mg, Amersham–Searle) in 0·20 ml 70% EtOH. The cultures were incubated at 24–26° for 30 days on a shaker.[10]

Extraction procedure. The tissues were removed from the medium by vacuum filtration, washed with 0·01 M phosphate buffer pH 6·0, and dried for 48 hr at 50°. The dried tissues were extracted with $CHCl_3$ in a Soxhlet for 24 hr. Following this extraction the air dried cells were acid hydrolyzed for 4 hr at 102–104° using 25 ml 2N HCl/2 g of tissue. The hydrolyzed cells were washed to neutrality with H_2O, dried for 24 hr at 60°, and extracted for 24 hr with $CHCl_3$ in a Soxhlet. The pooled medium was extracted 3 × equal vol. of $CHCl_3$.

Isolation and identification of metabolites. The post acid hydrolysis $CHCl_3$ extract of the tissue was chromatographed on 150 g silica gel, eluting successively with 2000 ml heptane–EtOAc (5:2), 500 ml heptane–EtOAc (1:1), 300 ml EtOAc, 300 ml EtOAc-MeOH (5:1), 400 ml EtOAc–MeOH (1:1), and 500 ml MeOH. All radioactivity appeared in the first 2000 ml of eluate which was collected in 10 ml fractions. All fractions were evaporated to dryness under N_2, redissolved in 2·0 ml CH_2Cl_2 MeOH (3:2), and aliquots removed for ^{14}C counting.

Two major radioactive peaks, A and B, were obtained, and the collection tubes corresponding to each were pooled. The two peaks were examined by TLC and the radioactivity associated with peaks A and B were found to cochromatograph with 5α-pregan-3β,20β-diol (III) and 5α-pregnan-3β-ol-20-one (II), respectively, on silica gel H (Brinkman) plates in $CHCl_3$–MeOH–H_2O (188:12:1), CH_2Cl_2–MeOH (97:3), and heptane–EtOAc (5:2) (developed 7 ×). The last system is capable of separating the α and β isomers of pregnanolone and pregnandiol. Routinely 6000–10 000 cpm were applied to each spot on the TLC plates. The reference standards that were developed with the two metabolites were located by exposing the TLC plates to iodine vapors. The location of the standard was marked on the plates, the iodine was allowed to evaporate and the areas corresponding to the reference standards were transferred to counting vials with the aid of a razor blade. The remainder of each developed radioactive column on the plate was divided into 1 cm zones and each was transferred to a counting vial. Toluene counting solution was added to all samples, and each was counted in a Beckman LS-100 liquid scintillation counter. All samples were counted for 20 min with a ^{14}C background of 10–12 cpm being routinely obtained.

The possibility that some of the radioactivity associated with the 5α-pregnan-3β-ol-20-one of peak B might be due to the formation of 5-pregnen-3β-ol-20-one which has the same *Rf* values was eliminated by reaction with *p*-nitroperbenzoic acid.[8] Only pregnenolone will form an epoxide. Upon adding pregnenolone to an aliquot of the peak B material and forming the epoxide, no radioactivity co-chromatographed with it upon TLC.

The two metabolites were further characterized by co-crystallization to constant specific activity with the authentic non-radioactive compounds. The specific activity of triplicate samples was determined following each recrystallization (Table 2).

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[10] B. KAUL and E. J. STABA, *Lloydia* **31**, 171 (1968).
[11] M. P. MORRIS, B. A. ROARK and B. CANCEL, *Agric. Food Chem.* **6**, 856 (1958).

Key Word Index—Dioscorea deltoides; Dioscoreaceae; biosynthesis; steroid; progesterone; 5α-pregnan-3β,20β-diol.