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SHORT COMMUNICATION

CONVERSION OF CORTISOL TO CORTISONE BY A HIGHER PLANT

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Abstract—After administration of cortisol-4-^{14}C to a *Mallotus paniculatus* plant, radioactive cortisone was isolated by chromatography and shown to be radiochemically pure by crystallization to constant specific activity.

INTRODUCTION

Mallotus paniculatus (Euphorbiaceae) is one of the few plants that contain steroids oxygenated at the 11-position. Among the cardenolides isolated from the seeds of this plant were 11-keto-uzarigenin and mallogenin (11β-hydroxyuzarigenin),[1] which suggests the existence of an enzyme system mediating oxidation and reduction at the 11-position. While animals and microorganisms are known to carry out this reversible reaction, such an enzyme system has not been found in higher plants heretofore. We have now obtained evidence that *M. paniculatus* can oxidize exogenously supplied cortisol to cortisone.

RESULTS AND DISCUSSION

Although most of the cortisol-4-^{14}C administered to the plant was recovered unchanged, cortisone was isolated by chromatography and demonstrated to be radioactive by crystallization to constant specific activity and conversion to cortisone acetate of the same molar specific activity (Table 1). The fraction of cortisol-4-^{14}C converted into cortisone was 0·42 per cent. Although cortisone and cortisol have never been isolated from plants, the reversible oxidation-reduction of other 11-oxygenated steroids most probably does take place naturally in higher plants, such as *Mallotus paniculatus*. However, so far, we have been unable to demonstrate 11-oxygenation in *M. paniculatus* by administration of progesterone-4-^{14}C, although it seems likely that this reaction must also occur in higher plants.

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[1] K. D. ROBERTS, E. WEISS and T. REICHSTEIN, *Helv. Chim. Acta* 49, 316 (1966).

TABLE 1. RECRYSTALLIZATION OF CORTISONE AND CORTISONE ACETATE TO CONSTANT SPECIFIC ACTIVITY*

Compound	Solvent used for crystallization	Counts/min/μmole†
Cortisone		304 ± 14
	Benzene–methanol	261 ± 12
	Benzene–methanol	220 ± 12
	Benzene–methanol	212 ± 10
	Hexane–acetone	204 ± 10
Cortisone acetate	Methanol	200 ± 9
	Hexane–acetone	209 ± 9

* Portions of 0·2 mg or less were plated from solution on ringed planchets over an area of 12·7 cm² and counted in duplicate on a Beckman Widebeta II instrument. Counter efficiency was 34 per cent and background was 4 cpm.

† 90 per cent confidence level.

EXPERIMENTAL

Techniques for TLC were as described previously.[2] All chromatograms were run on silica gel G plates purchased from Analtech, Inc., Wilmington, Delaware. Aliquots of radioactive samples were counted on planchets of infinite thinness under a gas-flow detector (see Table 1, legend, for details). Cortisol-4-^{14}C (53·8 μc/μM) was purchased from New England Nuclear Corporation. A portion of this material was subjected to TLC with CH_2Cl_2–methanol (9:1) and then scanned for radioactivity. A single radioactive peak corresponding to cortisol was observed. At the level of sensitivity used, as little as 0·05 per cent of cortisone could have been detected.

Cortisol-4-^{14}C was applied as a solution in ethanol–dimethylsulfoxide (9:1) to the leaves of a potted *Mallotus paniculatus* plant, 3 months old, in doses of $3{\cdot}50 \times 10^6$ cpm. A total of nine such treatments were given twice weekly. 4 days after the last treatment, the plant was harvested, frozen in liquid N_2, and lyophilized. The dried material (2·9 g) was homogenized, extracted and hydrolyzed by the methods described previously,[3] to yield 115 mg ($1{\cdot}95 \times 10^7$ cpm) of hydrolysate. TLC with CH_2Cl_2–methanol (9:1) showed a major peak corresponding to cortisol (R_f 0·31), a minor peak corresponding to cortisone (R_f 0·39), and an unidentified minor peak (R_f 0·54).

One-fifth of the hydrolysate was then subjected to preparative TLC in the same system and the cortisone zone was removed and eluted to give 2·7 mg ($1{\cdot}90 \times 10^5$ cpm). This material, along with 100 μg of cortisone as carrier, was further purified by preparative TLC with EtOAc–H_2O (99:1), to yield 0·6 mg ($3{\cdot}81 \times 10^4$ cpm), which was radiochromatographically homogeneous by TLC with CH_2Cl_2–acetone (7:3) and, after acetylation, with CH_2Cl_2–methanol (93:7).

About one-half of this material was diluted with 21·0 mg of cortisone and crystallized as shown in Table 1. After the second crystallization, 6·6 mg more of cortisone was added and a correction was made for this additional dilution in the subsequent specific activities. When constant specific activity was attained, the cortisone was acetylated and further crystallized.

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[2] R. D. BENNETT and E. HEFTMANN, *Phytochem.* **5**, 747 (1966).
[3] R. D. BENNETT, H. H. SAUER and E. HEFTMANN, *Phytochem.* **7**, 41 (1968).