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A Method for the Preparation of Labelled Gossypol by the Incorporation of ^{14}C Acetate[1]

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SUMMARY

Carbon-14 labelled gossypol has been prepared by incorporating 1,2-^{14}C acetate in the growth medium of cold shocked cotton (*Gossypium arboreum*) seedling roots. Unlabelled gossypol was added during the purification procedure to aid crystallization; thus, from 350 seedling roots, 25 uCi sodium (1,2-^{14}C) (45-60 mCi/mmol) acetate and 800 mg of unlabelled gossypol; 350 mg of labelled gossypol (1900 dpm/mg) was isolated.

Key Words: Cotton, gossypol, ^{14}C acetate.

INTRODUCTION

A principal pigment found in plants of the genus *Gossypium* (Malvaceae) is known as gossypol.[1] (Fig. 1.) Its chemistry and biological activity have been reviewed[1,2].

Gossypol has been isolated from seeds, rootbark, and subepidermal glands of cotton plants.[1,3] Gossypol, a sesquiterpenoid dimer is biosynthesized from acetate via the isoprenoid pathway. The incorporation of acetate as shown in figure 1 has been demostrated from ^{14}C- and ^{13}C- acetate studies.[4,5]

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Although the biochemistry and toxicology of gossypol were extensively investigated during the 60's and 70's,[1,2] the compound continues to be of interest because of its diverse biological activity. In our research, radiolabelled gossypol was needed for studies on detoxification in insects and microorganisms. It would also be useful in studies of toxicity to mammals,[2]

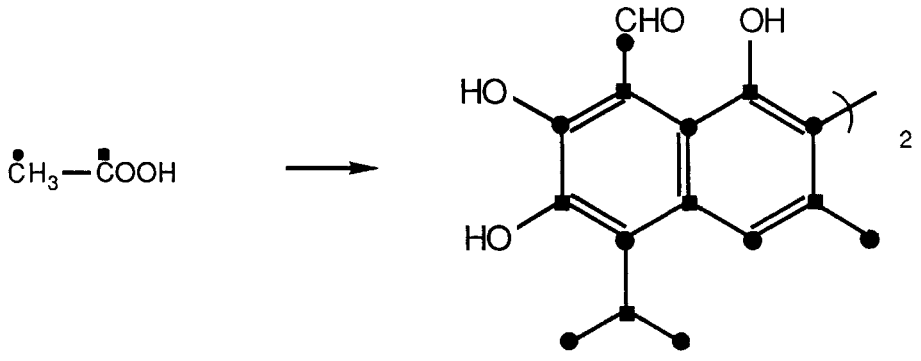

Figure 1. Labelling of gossypol prepared from 1-^{14}C-acetate, 2-^{14}C-acetate.

activity as a male antifertility agent in humans[6], toxicity to cotton insect pests including *Heliothis spp.*[7,8] and thurberia weevil,[9] and toxicity to the cotton plant pathogen, *Verticillium dahliae.*[3]

The present paper presents a convenient preparation of crystalline, homogeneously radiolabelled ^{14}C gossypol acetic acid complex from cotton roots which can be used in metabolism and bioactivity studies. The compound was isolated as the acetic acid complex because of its stability compared to the pure compound.

EXPERIMENTAL

Cotton seeds (*Gossypium arboreum,* var CJ-73) were heat treated at 80°C for 90 seconds and chilled in ice water for 20 seconds. Treated seeds were placed between 2 germination paper sheets, rolled in a wax paper sheet, placed in a plastic beaker containing the fungicide benomyl in deionized water (1 gm/4L)[10],

and incubated in the dark successively at 28°C for 4 days, and 10°C for 4 days. Approximately 700 seedlings were then severed at the transition zone, and the root portions were laid horizontally in a glass dish (33 X 22 X 5 cm deep) containing an aqueous (100 ml) solution of sucrose (1 gm), sodium (1-^{13}C) acetate (100 mg) (93 atom %), 50 uCi sodium (1,2-^{14}C) acetate (45-60 mCi/mmol), and 0.02 M potassium phosphate buffer at pH 6.5. The roots were evenly spread in the dish, covered with plastic wrap, and incubated in the dark at 30°C for 36 hrs. Upon removal from the solution, the roots were washed with ethyl alcohol (1250 ml) and the ethanol extract was filtered and reduced to 350 ml by rotary evaporation under vacuum. One half of this extract was concentrated (4 ml), and methyl ethyl ketone (2 ml), 4 drops acetic acid, and gossypol acetic acid complex (800 mg) were added. The solution was warmed to dissolve the crystals, and the mixture was then allowed to cool to room temperature and held at 0°C for 24 hrs. The crystals were separated from the mother liquid, and were recrystallized 3 times as described above to give gossypol acetic acid complex (m. p. 172 - 178.5) (350 mg, 1900 dpm/mg). Recrystallizations did not alter the specific activity. The gossypol could be further purified by thin-layer chromatography on Baker silica gel G/HR (hexane : ethyl acetate; 65 : 35), and crystallization as above (m. p. 190, authentic sample 188 - 192).

RESULTS AND DISCUSSION

Gossypol, although the major terpenoid aldehyde produced by cotton roots, is found in extracts from this tissue as a complex mixture of related terpenes[5] including gossypol-6-methyl ether, gossypol-6,6'-dimethyl ether, hemigossypol, and hemigossypol-6-methyl ether.[3] Chromatographically these compounds behave similarly to gossypol and purification of a labile compound like gossypol from such a complex matrix is difficult. Addition of the pure terpenoid allows direct crystallization of gossypol as its acetic acid complex from the crude extract; thus manipulation of the radiolabelled extract is minimized. Others have reported a different method for preparing ^{14}C labelled gossypol (3428 dpm/mg) from cotton roots, using 0.4 mCi/m mole sodium 1-^{14}C acetate[10]. However, these

results are based on values obtained from HPLC of the crude extract. This radiolabelled gossypol is probably contaminated with the mono-and dimethyl ethers of gossypol;[5] a crystalline product was not reported.

A procedure for preparing tritium labelled gossypol has also been reported[11]. The tritium is incorporated specifically at the 4,4' position and is therefore unsuitable for metabolism studies in which that position is oxidized.

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