SYNTHESIS OF DEUTERATED AND TRITIATED APOGOSSYPOL

Larry J. Powers*
Department of Molecular Biology
College of Pharmacy
University of Tennessee
Center for the Health Sciences
Memphis, TN 38163

Preston H. Dorsett
Department of Microbiology
College of Basic Medical Sciences
University of Tennessee
Center for the Health Sciences
Memphis, TN 38163

SUMMARY

Apogossypol with deuterium or tritium in the 8 and 8' positions can be synthesized by the base catalyzed deformylation of gossypol. The position of the labeling is established by the pmr spectrum of the material derived from the reaction of deuterium oxide and sodium deuteroxide. The specific activity of the tritiated material prepared from tritium oxide and sodium hydroxide is 110m Ci/m Mole. The methods described are also useful for the synthesis and storage of small quantities of unlabeled apogossypol.

Key words: apogossypol, gossypol, tritiated, deuterated, deformylation.

Apogossypol (1,1',6,6',7,7'-hexahydroxy-5,5'-diisopropyl-3,3'-dimethyl-2,2'-binaphthalene) is formed by the base catalyzed deformylation of gossypol (8,8'-dicarboxaldehyde-1,1',6,6',7,7'-hexahydroxy-5,5'-diisopropyl-3,3'-dimethyl-2,2'-binaphthalene), a major constituent of the pigment gland of cotton seed[1]. As part of a study[2] of the antimicrobial properties of apogossypol, a sample of radioactive material was needed for in vitro experiments. Although ^{14}C-gossypol derived by a biosynthetic route has been previously described[3], the tritiated compound has not been reported. This paper describes a convenient synthesis of tritiated apogossypol from tritium oxide. The position of labeling was established by the pmr spectrum of the compound derived from the reaction of gossypol with deuterium oxide and sodium deuteroxide. The loss of the tritium label from the apogossypol on standing in aqueous solution was also investigated.

*Address correspondence to L. J. Powers, Diamond Shamrock Corporation, T. R. Evans Research Center, P. O. Box 348, Painesville, Ohio 44077.

0362-4803/80/0217-0309$01.00

Received November 22, 1978
Revised March 9, 1979

The synthetic procedure used is essentially that of Clark[4]. We have modified it to allow for conducting the reaction in an inert atmosphere and for storing the apogossypol, which undergoes facile air oxidation, in evacuated glass tubes. The vessel used for the reaction is 8 mm OD (1 mm wall) x 250 mm pyrex glass tubing. Gossypol, contained in a small glass tube, is added to a frozen solution of NaOH. The purpose of the glass tube is to quantitatively transfer the gossypol to the bottom of the reaction tube. Freezing the NaOH solution prevents reaction with the gossypol prior to removing the oxygen from the tube. The tube is sealed under vacuum and heated until the effervescence within the tube ceases. The reaction mixture is amber in color at this point. The product of the reaction is isolated and crystallized from ethanol and water, to exchange the deuterium or tritium of the phenolic groups. The product is harvested by centrifugation and dried in vacuo. The evacuated tube is sealed and the white to beige apogossypol stored at -20°C. No visible change is observed for up to 12 months in the apogossypol stored in this manner.

The only difference in the pmr spectrum of apogossypol prepared from $NaOD/D_2O$ as compared to that prepared from $NaOH/H_2O$ is the absence of the signals for the 8 and 8' hydrogens in the spectrum of the deuterated material (Table 1). The assignments of the pmr signals listed in Table I are based on comparison of the spectra of gossypol, apogossypol and apogossypol hexaacetate. Analyses of the pmr spectrum of gossypol have been published previously[5]. The pmr spectra establish that the deuterium incorporation (90%) is at the 8 and 8' positions and that the phenolic deuterons have been exchanged (90%) for protons during the purification.

Tritiated apogossypol generated from tritium oxide using this procedure had a specific activity of 110m Ci/m Mole. Comparison of the thin layer chromatogram (Figure 1) of ^3H apogossypol which is either a freshly prepared solution or stored in aqueous solution for six days at 5° C, establishes that the tritium is exchanged under these storage conditions. In the illustrated experiment, an 86% loss of non volatile reactivity was observed. Although tritium exchange with solvent does occur, the tritiated apogossypol is sufficiently stable, so that qualitative in vitro experiments on the uptake and cellular localization of apogossypol can be conducted[6].

The reasons for observations of a non-symmetrical thin layer chromatogram (Figure 1) were not investigated. The most likely explanations for this observation are; (1) air oxidation of the apogossypol during chromatography, (2) surface catalyzed decomposition of the apogossypol during chromatography and (3) the generation of more than one component during the deformylation reaction. We feel that the later possibility is unlikely based on the observed pmr spectra of the protonated and deuterated compounds as well as the prior observations on this reaction by ourselves and other investigators[1,4].

We feel that the above observations establish that base catalyzed deformylation of gossypol leads to incorporation (in 90% regiospecificity) of deuterium in the 8 and 8' positions. It is also evident that the tritiated apogossypol generated under these conditions is labile to exchange in aqueous media. For this reason the 8,8'-^{3}H-apogossypol is only suitable for short term, qualitative, in vitro investigation of the cellular localization of apogossypol. The experimental procedure described is also convenient for the generation of small (1-50 mg) amounts of apogossypol and the storage of this material in vacuo.

Experimental

The preparations of the deuterated, protonated and tritiated apogossypol are identical with the one exception that a commercial 40% NaOD/D_2O solution (Aldrich) was used in the preparation of the deuterated compound. Tritiated water (ICN, 1Ci/mL) was used to generate the ^{3}H-apogossypol. All aqueous solutions were degassed prior to use. Tlc was determined on EM Reagents precoated silica gel F-254 plates (5x10 cm) using C_6H_6 : EtOAc : Formic Acid (10:4:1) as the eluting solvent. Pmr spectra were recorded on a Varian 80 MHz spectrometer in DMSO-d_6.

Preparation of Apogossypol

NaOH (100 mg 2.5 m mol) is placed in a reaction tube. Water (0.25 mL, 14 mmol) is added and the tube cooled in ice until the NaOH is dissolved. The 40% NaOH is then frozen (dry ice, acetone bath). Gossypol (30 mg, 0.058 mmol) in a small tube is added and the tube evacuated and flushed with argon. The flushing is repeated and the tube evacuated (to ca. 1 Torr). The

tube is sealed and, after warming to room temperature, placed in a boiling water bath for 30 minutes. The tube is allowed to cool to room temperature and then frozen (dry ice, acetone bath). A solution of 0.15 mL of conc. H_2SO_4 in water (0.35 mL) is added and the sample allowed to warm. Mixing the reaction mixture on a vortex mixer, provides a white precipitate. Ether (0.5 mL) is added and the tube again mixed on the vortex mixer. The ether solution is transferred using a Pasteur pipet to a small culture tube, and the aqueous solution extracted again with ether. The combined ether layers are dried (Na_2SO_4), transferred to another culture tube, and evaporated under a stream of argon. The residue is dissolved in ethanol (0.25 mL) and the solution transferred to an 8mm reaction tube. The culture tube is washed with another 0.25 mL of ethanol, and the wash solution combined with the ethanol solution. Water is added to the combined ethanol solutions to precipitate the product. The resulting precipitate is harvested by centrifugation. The mother liquor is decanted, and the residue dried in vacuo.

Acknowledgments

This work was supported by the United States Department of Agriculture with funds made available through Cotton Inc. The authors express their appreciation to Dr. Richard F. Sprecher of Memphis State University for determining the pmr spectra.

References

1. Adams, Roger, Geissman, T. A. - Chem. Rev. 60:555 (1960).
2. Dorsett, P. H., Kerstine, E. E., and Powers, L. J. - J. Pharm. Sci. 64:1073 (1975)
3. Smith, F. H. - J. Amer. Oil Chem. Soc. 51:410 (1974).
4. Clark, E. P. - J. Biol. Chem. 78:159 (1928).
5. Stipanovic, R. D., Bell, A. A., and Howell, C. R., J. Amer. Oil Chem. Soc., 50:462 (1973).
6. Dorsett, P. H., and Powers, L. J., unpublished results.

TABLE I

PMR SPECTRA OF APOGOSSYPOL AND DEUTERATED APOGOSSYPOL

Assignment	8,8'-^{2}H Apogossypol	Apogossypol
8-H	-	7.35
OH	7.35 (s)	7.35 (s)
OH	9.69 (s)	9.69 (s)
OH	8.07 (s)	8.07 (s)
4-H	7.56 (s)	7.57 (s)
CH_3	1.93 (s)	1.94 (s)
$C\underline{H}(CH_3)_2$	3.80 (sept)	3.80 (sept)
$CH(C\underline{H}_3)_2$	1.44 (d)	1.44 (d)

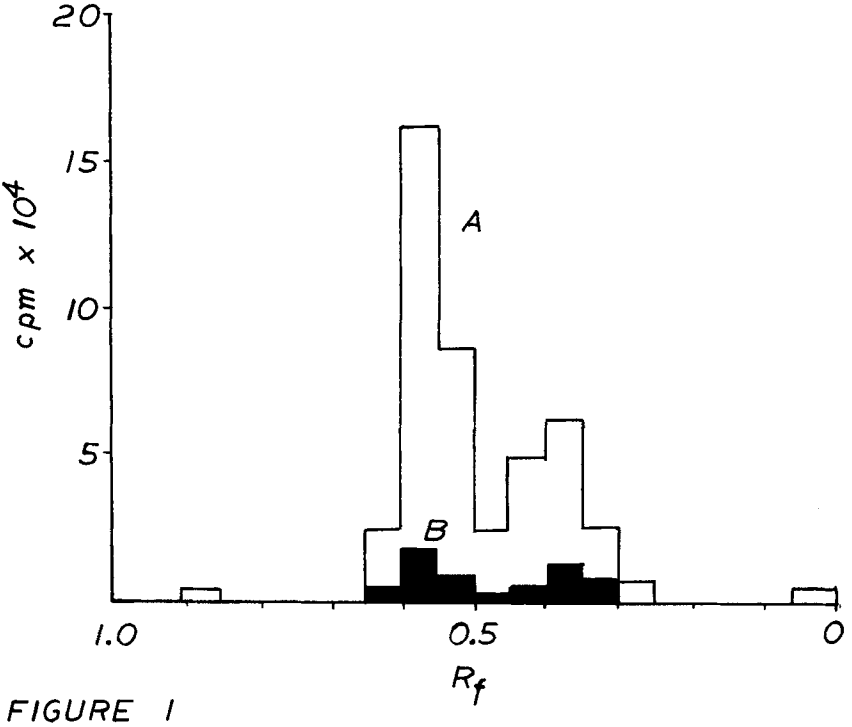

FIGURE 1