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SYNTHESIS OF DEUTERATED AND TRITIATED GOSSYPOL

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Summary

We report here the synthesis of deuterated and tritiated gossypol by exchange with labelleled water using trifluoroacetic acid as the catalyst. In the case of D_2O in tetrahydrofuran, the exchange is complete in 45 minutes at 75°C as determined by a ^1H-NMR spectrum.

Key Words: Gossypol, deuterium, tritium, acid catalyzed exchange.

INTRODUCTION

Gossypol (1) is one of several terpenoid aldehydes found in pigment glands of cotton (Gossypium) and seven other genera of the tribe Gossypieae.[1] Gossypol is toxic to monogastric animals, and its presence in cottonseed meal prompted numerous toxological studies during the first half of the twentieth century.[2,3] The recognition of gossypol as a compound that confers plant resistance to insect attack[4], and its activity as a male antifertility agent in humans[5] has stimulated renewed interest in this compound. For various biochemical studies, we required, labeled gossypol, and we here report a simple, inexpensive method for labelling gossypol by substituting deuterium or tritium for protons on the aromatic ring.

EXPERIMENTAL

General: Gossypol was obtained from Dr. Walter A. Pons, Jr. (deceased), Agricultural Research Service, USDA, Southern Regional Research Center, New Orleans, LA. Tritiated water (1 Ci/mL) was obtained from New England Nuclear. ^1H-NMR spectra were recorded in $CDCl_3$ on a JEOL FX-90Q operating at 89.55

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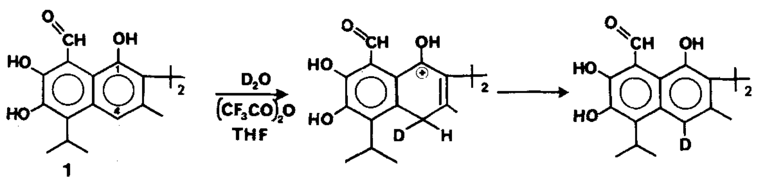
MHz. Chemical shifts are reported in ppm upfield from TMS [using $CDCl_3$ (δ. 7.28) as an internal standard]. MS were obtained on a VG Analytical Model 70-250.

Gossypol-d_4-d_4': Gossypol, as the acetic acid complex (10 mg), was dissolved in 400uL of THF in a 5mL test tube by gentle warming. D_2O (1000uL) was added and the solution was warmed again to dissolve any precipitated gossypol. After cooling to room temperature, trifluoroacetic anhydride (100 uL) was added carefully over 1 min. Air was expelled with a stream of N_2, and the tube was lightly stoppered and placed in a hot water bath (75°C) for 45 minutes. The green solution was cooled in ice and 400 uL of H_2O was added. Gossypol crystallized as dark green crystals. The crystals were washed with H_2O (3x 1mL) and dried in a vacuum dessicator (yield 8 mg, 80%, m.p. 177-180°C). MS: m/z (% of base peak) 520 (M^+,0.6), 504(0.5), 503(1.7), 502($M-H_2O$,4.0), 501(1.1), 500(0.7), 488(0.5), 487(2.4), 486(9.4), 485(39.3), 484($M-2H_2O'$, 100), 483(26.8), 482(4.6), 469($M-CH_3$, $2H_2O$,38.8). ^1H-NMR: δ 1.58[12H, d, $CH(C\underline{H}_3)_2$], 2.18 (6H, s, $Ar-CH_3$), 3.94 [2H, sept, $C\underline{H}(CH_3)_2$], 5.74 (2H, s, C_1-OH), 6.47(2H,s, C_6-OH), 11.17 (2H, s, CHO), 15.00 (2H, s, C_7-OH). UV: λ_{max} (ϵ): 238(67,500), 290(25,400), 378(14,200) nm.

Gossypol-t_4-t_4': The exchange reaction was performed as above using 50 mg of gossypol, 2.0mL of THF, 500uL of tritiated water (1 Ci/mL) and 500uL of trifluoroacetic anhydride. The product, washed and dried as above, had a specific activity of 8.2mCi/mmole.



RESULTS AND DISCUSSION

The gossypol obtained from the D_2O exchange was pure as determined by its m.p. and its UV spectrum [for gossypol, UV: λ^{MeOH} (ϵ) 238(63,200), 290(23,850), 378(13,450) nm]. ^1H-NMR agreed with the published spectrum[6] except the peak for the aromatic protons at δ7.81 (s) was absent.

The exchange reaction of the aromatic protons in gossypol is the result of the enol-keto equilibrium of the phenol groups at C-1, C-1′ and perhaps C-6, C-6′. Gossypol tritiated at the C-4, C-4′ positions should provide an excellent probe in biological studies because protons at these positions exchange very slowly at or near neutral pH and at temperatures suitable for biochemical studies. The presence of the appropriate number of protons on all phenolic hydroxyl groups in the deuterium experiment, as determined by integration of the ^1H-NMR spectrum, indicated that these protons are rapidly exchanged with water during the precipitation and washing process. Deuterium or tritium found on the molecule is therefore due to substitution on the aromatic ring.

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