PREPARATION OF TRITIUM-LABELED DIOSGENIN, ESMILAGENIN and TIGOGENIN

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SUMMARY

The synthesis of ^{3}H -diosgenin, ^{3}H -esmilagenin and ^{3}H -tigogenin (three potential hypocholesterolemic agents) was accomplished by low pressure hydrogenation of commercial (Sigma D 1634) diosgenin with ^{3}H -gas. The mixture of ^{3}H materials obtained was separated by silica gel G-60 column chromatography by use of a benzene: ethyl acetate (8:2 v/v) solution. Purification of each sapogenin was by TLC chromatography.

Key Words: ^{3}H -Diosgenin, ^{3}H - Esmilagenin, ^{3}H -Tigogenin, ^{3}H -hydrogenation, Modified Wilzbach labeling with reduction.

INTRODUCTION

Diosgenin, esmilagenin and tiogenine are found in the Mexican Yam (Dioscorea tokoro, Makino). This Yam and its extracts have been suggested as possible hypocholesterolemic agents, because of their structures and poor intestinal absorptions (1,2). It was necessary to prepare these three compounds labeled to study their intermediary metabolism. The synthesis was the ^{3}H -hydrogenation at low pressure of diosgenin (Sigma D 1634) (3β-Hydroxy-5-spirostene; [25R], 5-Spirostene-3β-ol). (See Figure 1). The hydrogenation with tritium gas was carried out in acetone solution. Commercial diosgenine which contained all three of the compounds was used. The ^{3}H-labeling was carried out by a modified Wilzbach reaction (3). This reaction yielded all three products tritium labeled. The pre-labeled mixture contained 98.5% diosgenin, 1% esmilagenine, and 0.5% tigogenine. After the Wilzbach labeling procedure the mixture contained 56% diosgenin, 28% esmilagenin and 16% tigogenin by trition assay. The mixtures were separated by column chromatography.

EXPERIMENTAL AND RESULTS

To 10 mg. of recrystallized diosgenin (Sigma D-1634) (m.p. 204°-206°; $[\alpha]_D^{25}$ -129° in $CHCl_3$) dissolved in 25 ml of acetone with 100 mg. of platinum

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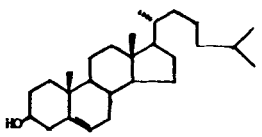
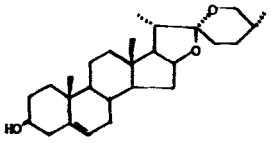
- 5% on charcoal catalyst (K and K - 17845); was slowly added with shaking 30 curies of $^{3}H_{2}$ -gas (New England Nuclear) in a closed system. After 24 hrs of agitation the reaction was stopped. (Different batches using varying amounts of tritium, diosgenine and catalyst were tried: this ratio was the best). The solution was filtered and the acetone was evaporated. The obtained mixture was then boiled for 1 hour with 50 ml of 10% KOH to remove exchangeable ^{3}H. At this point the total ^{3}H -activity recovered from the mixture was 260 mCi. Some was in a non-steroidal portion. The mixture was then extracted three times with ether. The ether solution was evaporated to dryness and the solids redissolved in a few drops of benzene and this solution added to a silica gel G-60 chromatography column (2.5 x 50 cm) (Bio-sil A, 100 -mesh-Bio-Rad Lab, Richmond, CA.). A solvent system (100 ml), benzene:ethyl acetate (8:2 v/v) was used to elute the column. Test-tubes each with 1 ml individual fraction was assayed for ^{3}H - radioactivity. The activity was only found in three groups of test-tubes (13-18; 25-36; 56-65). The test- tube contents of each group was pooled and the solvent evaporated. The specific activity for diosgenine was 1.09 mCi/mmole; 3.00 mCi/mmole for esmilagenin and 1.77 mCi/mmole for tigogenin. Further purification for each group was carried out by gas liquid chromatography, the column was GP 10% DEGS-PS on

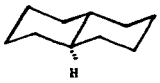


CHOLESTEROL



DIOSGENIN

"CIS" - "TRANS"
CONFIGURATION



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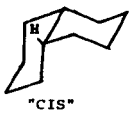

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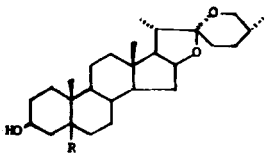

DIHYDRODIOSGENIN

R = 5β = ESMILAGENIN

R = 5α = TIGOGENIN

80/100 Supelcoport (Supelco, Inc. Bellefonte, PA). (310° injector, 280° oven, 20 ml/min N_2); the sterols showed elution peaks at 8.21, 8.78, and 9.09 min, for esmilagenin, diosgenin, and tigogenin, respectively. Purification using silica-gel TLC plates, (G-type 250 microns-Caltech, Newark, DE) was made for each one of the labelled sapogenins with a solvent system of benzene:ethyl acetate: (80:20 v/v), and the RF values were 0.72, 0.64, and 0.60 for esmilagenin, diosgenin and tigogenin, respectively.

It is known that Wilzbach method (3) has a tendency to yield reduction of unsaturated bonds. It was the intention of the investigators to increase the yield of this reduction by use of Pt-C. In this manner reasonable amounts of the rare tigogenin and esmilagenin could also be obtained. The amount of chemical sterol was determined by spectra (4) and charring (5).

Radioactivity was determined by use of a Packard Tri-Carb 460 Liquid Scintillation Spectrometer using Bioflour (New England Nuclear) as the counting medium with pulse height discrimination for ^{3}H. Counting was for a period long enough to ensure a maximum error of $\pm$ 2%. The final specific activity obtained for the highly purified diosgenin was 0.97 mCi/mmole; 2.10 mCi/mmole for esmilagenin and 1.25 mCi/mmole for tigogenin.

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