Journal of Labelled Compounds — April-June — Vol. V, No. 2

Synthesis of kryptogenin-26-^{14}C and diosgenin-26-^{14}C

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Received on the 6th March, 1969

SUMMARY

*Kryptogenin-26-^{14}C (**13**) was synthesized from dihydrodiosgenin diacetate (**1**). The radioactive carbon atom was introduced by the Arndt-Eistert method, and the extra carbon atom was then removed by a carboxy-inversion reaction. Diosgenin-26-^{14}C (**14**) was prepared by reduction of kryptogenin-26-^{14}C.*

INTRODUCTION.

For our work on the biosynthesis and metabolism of steroidal sapogenins in plants, radioactive kryptogenin (**13**) would be useful for several reasons. First, it is frequently found as a companion of diosgenin (**14**) in plants and could be an intermediate in the biosynthesis [1] of the latter from cholesterol. Furthermore, kryptogenin can be converted chemically [2] to three other possible precursors in the biosynthesis of diosgenin : 16β-hydroxycholesterol, 26-hydroxycholesterol, and 16β,26-dihydroxycholesterol. Finally, labeled diosgenin for metabolic experiments can be made by chemical reduction of kryptogenin. Accordingly, we have now synthesized kryptogenin-26-^{14}C and diosgenin-26-^{14}C.

The steps used in the synthesis are shown in Figure 1. Both the kryptogenin-26-^{14}C and diosgenin-26-^{14}C were shown to be radiochemically pure by dilution with carrier material and crystallization to constant specific activity (Table 1).

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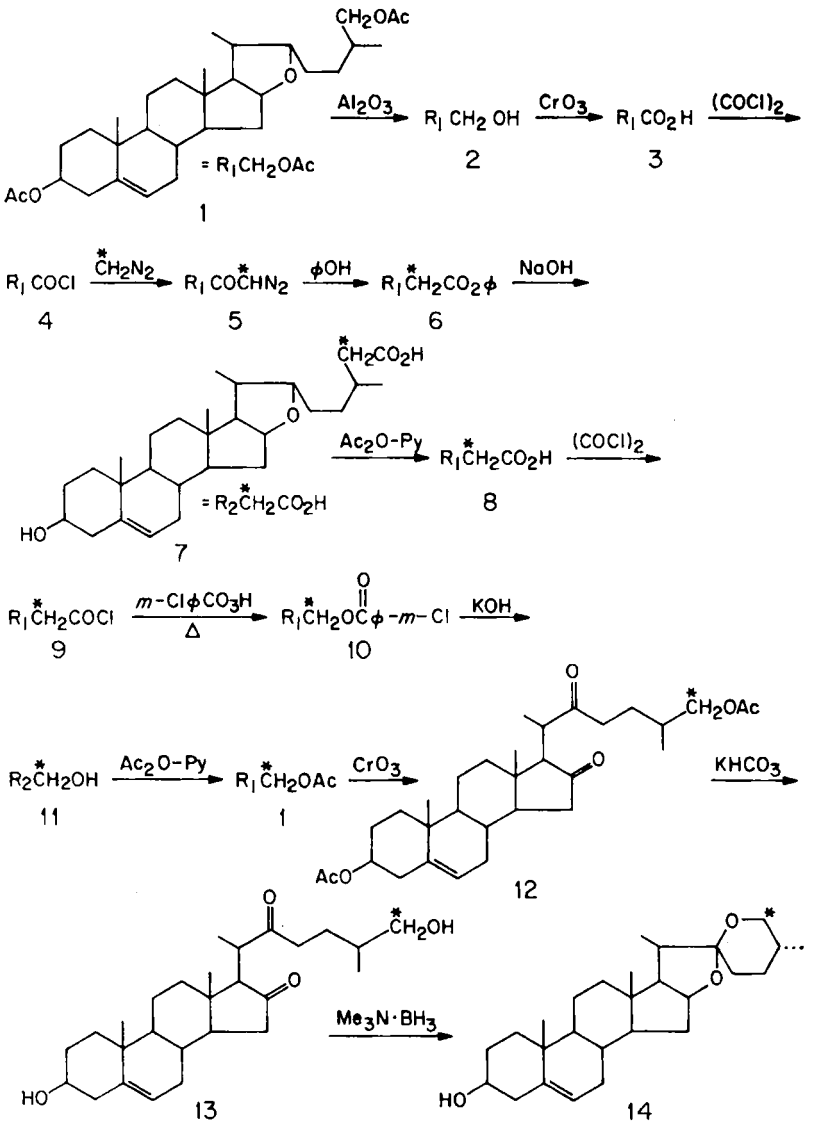
** A laboratory of the Western Utilization Research and Development Division, U. S. Department of Agriculture. Work conducted under a cooperative agreement with the California Institute of Technology.



EXPERIMENTAL.

Δ^5-Furostene-3β,26-diol 3-monoacetate (2).

The primary acetoxy group of dihydrodiosgenin diacetate (**1**) was selectively hydrolyzed by the method previously used for dihydrotigogenin diacetate [3].

TABLE 1. Recrystallization of Kryptogenin and Diosgenin [a]

Compound	Solvent	Cpm/μmole [b]
Kryptogenin		276 ± 9
	Acetone	265 ± 9
	Ethanol	265 ± 9
Diosgenin		155 ± 7
	Acetone	150 ± 7
	Hexane-dichloromethane	151 ± 7

[a] Portions of 0.2 mg or less were plated from solution on ringed planchets over an area of 12.7 cm^2 and counted in duplicate on a Beckman Widebeta II instrument. Counter efficiency was 34 % and background was 1.5 cpm.

[b] 90 % confidence level.

A solution of 113 mg of dihydrodiosgenin diacetate [4] in 3 ml of hexane-benzene (1 : 1) was placed on top of a 14-g column of Grade I alkaline alumina*, which had been packed in hexane. The solution, followed by 10 ml of hexane-benzene (1 : 1), was allowed to run into the column, which was then closed at the bottom. After 90 h the column was eluted as follows (60 ml fractions) : *1*, 0 %; *2-3*, 5 %; *4-5*, 10 %; *6*, 20 %; and *7*, 50 % ethyl acetate in benzene. Thin-layer chromatography (TLC)** of the fractions with dichloromethane-acetone (9 : 1) showed that fractions 5-7 (76 mg) contained the desired product. This material was purified by preparative TLC with the same system to give 57 mg of chromatographically homogeneous **2**. An analytical sample, obtained by recrystallization from methanol-water, had m.p. 109.5-111°.*** Analysis for $C_{29}H_{46}O_4$: calcd., C 75.93 %, H 10.11 %; found, C 75.91 %, H 10.00 %.

An NMR spectrum**** showed identifiable peaks at 0.82 and 1.07 (18- and 19-methyl), 2.05 (CH_3COO—), 3.45 and 3.54 (—CH_2O—), and 5.35 (> C = CH—) ppm.

* Woelm, Eschwege, Germany. Reference to a company or product name does not imply approval or recommendation of the product by the U. S. Department of Agriculture to the exclusion of others that may be suitable.

** All chromatograms were run on Silica Gel G plates purchased from Analtech, Inc., Wilmington, Delaware.

*** All melting points were taken on a Kofler block and are corrected.

**** NMR spectra were taken on a Varian A-60A instrument in $CDCl_3$. Chemical shifts are indicated in ppm with $SiMe_4$ as internal standard.

*Δ^5-Furostene-3β-ol-26-oic acid 3-acetate (**3**).*

A solution of 44 mg of **2** in 2 ml of acetone was cooled to 0° C and treated with 90 μl of Kiliani's solution (prepared by dissolving 2.6 g of chromic acid in 2.3 ml of concd. H_2SO_4 and 7 ml of water). The mixture was kept at 0° for 10 min, and then a few drops of methanol were added to reduce the remaining chromic acid. The mixture was concentrated to 1.5 ml under a stream of nitrogen and, after addition of 2 ml of water, extracted with three 2-ml portions of benzene. The extracts were washed with 1 ml of 5 % NaOAc solution and 1 ml of water, combined, and evaporated. The residue was chromatographed on a 3-g column of silica gel (particle size 0.2-0.5 mm),* packed as a slurry in hexane-acetone (24 : 1) in a chromatographic tube of 14 mm diameter. Nonacidic material was eluted with 13 ml of 4 %, 13 ml of 10 %, and 8 ml of 20 % acetone in hexane. Elution with 60 ml of 20 % acetone in hexane then gave 36 mg of **3**.

A portion of the product was methylated with diazomethane, and the methyl ester was purified by preparative TLC with hexane-acetone (17 : 3). An analytical sample, obtained by recrystallization from methanol, had m.p. 104-105° C. Analysis for $C_{30}H_{46}O_5$: calcd., C 74.03 %, H 9.53 %; found, C 73.90 %, H 9.47 %.

An NMR spectrum showed identifiable peaks at 0.79 and 1.03 (18- and 19-methyl), 2.01 (CH_3COO—), 3.68 (—$COOCH_3$), and 5.41 (>C = CH—) ppm.

*Δ^5-Furostene-3β-ol-26-carboxylic acid-26-^{14}C 3-acetate (**8**).*

The Arndt-Eistert reaction, as modified by Wilds and Meador [5], was used to introduce the ^{14}C label into the 26-position.

A solution of 32 mg (0.068 mmole) of **3** in 800 μl of dry benzene was treated with a solution of 30 μl (45 mg, 0.35 mmole) of oxalyl chloride in 800 μl of dry benzene. The solution was allowed to stand for 40 min and then evaporated to dryness in vacuum. The residue was treated with 500 μl of benzene, and, after evaporation, 33 mg of the oily acid chloride **4** was obtained.

A solution of 29.6 mg (0.138 mmole) of N-methyl-^{14}C-N-nitroso-*p*-toluenesulfonamide** (0.5 mCi) and 140 μl of 2-(2'-ethoxyethoxy)ethanol in 800 μl of ether was treated with a solution of 8.6 mg of KOH in 30 μl of water. The tube was sealed with a rubber septum pierced by a 9-cm long piece of 18-gauge metal tubing. The other end of the tubing was immersed in a test tube containing 300 μl of ether, cooled to —10° C. The reaction tube was heated to 50° C, and the diazomethane-ether distillate was collected in the

* Brinkmann Instruments, Westbury, New York.

** New England Nuclear Corporation, Boston, Massachusetts.

receiver tube and then dried over a KOH pellet for 20 min. After addition of 7 μl of triethylamine to the diazomethane solution, it was treated slowly with a solution of the acid chloride **4** from above in 500 μl of ether-dichloromethane (9 : 1). The reaction mixture was kept at 25° C for 2 hr, then evaporated to dryness under nitrogen, taken up in 2 ml of benzene, and centrifuged to remove insoluble material. The latter was washed with another 2-ml portion of dry benzene. When the benzene solutions were combined and evaporated, 34 mg (6.40×10^7 cpm)* of product was obtained. Preparative TLC with dichloromethane-acetone (19 : 1) gave 8.2 mg of the oily diazoketone **5** (4.92×10^7 cpm). This material was heated at 160° C for 4 min with 30 mg of phenol and 30 μl of N,N-dimethyl-aniline. The product was taken up in 2 ml of dichloromethane and extracted with two 1-ml portions of 1*N* NaOH solution, two 1-ml portions of 2*N* HCl, and two 1-ml portions of water. The extracts were washed with 1 ml of dichloromethane and discarded. The two dichloromethane solutions were combined and yielded on evaporation 8.8 mg of the crude phenyl ester **6** (4.56×10^7 cpm). This material was treated with 1 ml of 0.4 *N* NaOH in 80 % methanol at 25° C for 16 hr under nitrogen. The solution was adjusted to pH 5 with acetic acid, and then concentrated under nitrogen to 0.5 ml, diluted with 1.5 ml of water, and finally extracted with three 2-ml portions of ethyl acetate. The extracts were washed with 1 ml of water and combined. After evaporation 8.4 mg of crude **7** (4.38×10^7 cpm) was obtained. For purification the acid was converted to the methyl ester by treatment with diazomethane. The ester was subjected to preparative TLC with dichloromethane-acetone (9 : 1).

The chromatographically homogeneous methyl ester (5.1 mg, 2.88×10^7 cpm) was hydrolyzed with NaOH, as above. The acid was then acetylated with 150 μl of pyridine and 150 μl of acetic anhydride at 25° C for 16 hr. The reaction mixture was subsequently diluted with 100 μl of water and heated at 100° C for 1 hr. The cooled mixture was diluted with 3 ml of water, acidified to pH 2 with 3*N* HCl, and extracted with three 2-ml portions of benzene. The extracts were washed with 1 ml of 5 % $KHCO_3$ solution and 1 ml of water, combined, and evaporated to give 4.4 mg of **8** (2.63×10^7 cpm).

A nonradiaactive preparation of **8** was methylated with diazomethane. An analytical sample of the methyl ester, obtained by recrystallization from methanol-water and then methanol, had m.p. 113.5-114.5° C. Analysis for $C_{31}H_{48}O_5$: calcd., C 74.36 %, H 9.66 %; found, C 74.45 %, H 9.59 %.

An NMR spectrum of the methyl ester showed identifiable peaks at 0.81 and 1.05 (18- and 19-methyl), 2.02 (CH_3COO–), 3.64 (–$COOCH_3$), and 5.37 (C = CH–) ppm.

* Aliquots of radioactive samples were counted on planchets at infinite thinness under a gas-flow detector (see Table 1, legend, for details).

*Dihydrodiosgenin-26-^{14}C diacetate (**1**).*

The carboxyl carbon atom of **8** was removed by the carboxy-inversion reaction of Denney and Sherman [6]. A large amount of unreacted **8** was always recovered from this reaction, even when rigourous precautions were taken to exclude moisture. It was therefore necessary to recycle this recovered material to obtain acceptable yields. It is noteworthy that although *m*-chloroperbenzoic acid is an excellent epoxidizing agent, protection of the Δ^5-double bond of **8** was not necessary provided that no excess of the peracid was used.

The radioactive **8** from above (0.0090 mmole) was dissolved in 100 µl of dry benzene and treated with a solution of 3 µl (4.5 mg, 0.035 mmole) of oxalyl chloride in 100 µl of dry benzene at 25° C for 40 min. The solution was evaporated to dryness in vacuum, treated with 200 µl of benzene, and again evaporated to dryness. The residual acid chloride **9**, together with 1.5 mg (0.0089 mmole) of *m*-chloroperbenzoic acid,* was cooled to 0° C, and a solution of 2 µl of dry pyridine in 200 µl of dry benzene, also cooled to 0° C, was added all at once. The mixture was agitated for a few seconds and then kept at 5° C for 10 min. The precipitate was removed by centrifuging and washed with 200 µl of benzene. The two benzene solutions were combined and evaporated under nitrogen. The residue was taken up in 200 µl of dichloromethane and extracted successively with 200 µl-portions of 5 % $KHCO_3$ solution, water, 1*N* HCl, and water. The extracts were washed with two 200-µl portions of dichloromethane and then discarded. The three dichloromethane solutions were combined and evaporated. The residue was taken up in 1 ml of isooctane and refluxed for 4 hr. The solvent was removed by evaporation, and the residual ester **10** was treated with 500 µl of 1*N* KOH in methanol-water-benzene (7 : 2 : 1) at 25° C for 16 hr under nitrogen. The solution was concentrated to 100 µl under nitrogen, taken up in 1 ml of dichloromethane, and extracted with five 300-µl portions of water. The extracts were washed with 500 µl of dichloromethane. When the two dichloromethane solutions were combined and evaporated, 2.2 mg of crude neutral product (1.28×10^7 cpm) was obtained.

The aqueous extracts from above were combined, acidified with acetic acid, and extracted with three 1-ml portions of ethyl acetate. The extracts were washed with 500 µl of water, combined, and evaporated. The acidic residue (1.7 mg, 1.05×10^7 cpm) was about 85 % **7**, as indicated by TLC with dichloromethane-methanol (23 : 2). This material was acetylated by the method used previously. Some of the product was lost during the workup. The remainder (1.4 mg, 8.6×10^6 cpm) was again subjected to the carboxy-inversion reaction (**8** → **11**) as above, and gave 0.6 mg (3.51×10^6 cpm) of neutral and 0.6 mg (3.65×10^6 cpm) of acidic material.

* 85 % *m*-Chloroperbenzoic acid was purchased from Aldrich Chemical Co. and purified [7].

The two neutral fractions were combined and acetylated by a vapor phase method [8, 9]. The product was purified by preparative TLC with dichloromethane-acetone (47 : 3) and 1.1 mg of **1** (5.96×10^6 cpm) was obtained. This material was identical to authentic **1**, the starting compound for the synthesis, by TLC in three systems.

*Kryptogenin-26-^{14}C (**13**).*

The method described by Marker *et al.*[10] for oxidation of dihydrotigogenin diacetate to dihydrokryptogenin diacetate at 100° C gave only a low yield of kryptogenin diacetate from **1** in our hands, even though the double bond was protected by bromination. We found that the maximum yield of kryptogenin diacetate was obtained by performing the oxidation at 50° C. At higher temperatures the reaction gave large amounts of acidic products, while at lower temperatures it was very slow.

The **1** from above was diluted with 3.0 mg of unlabelled **1**, dissolved in 100 µl of acetic acid, and treated with a solution of 1.5 mg of bromine in 20 µl of acetic acid for 5 min at 25° C. The solution was then evaporated to dryness in vacuum, and the residue was taken up in 100 µl of acetic acid and heated at 50° C, while a solution of 3 mg of chromic acid in 30 µl of 80 % acetic acid was added over a period of 2 hr. The excess chromic acid was subsequently destroyed by adding 2 mg of sodium bisulfite, and the acetic acid was removed under vacuum. After adding 200 µl of water to the mixture, it was extracted with three 200-µl portions of ethyl acetate. The extracts were combined and evaporated. The residue was taken up in 100 µl of acetic acid and debrominated by heating at 100° C with 5 mg of sodium acetate and 20 mg of zinc dust for 20 min. The acetic acid was evaporated, 200 µl of water added, and the mixture was extracted with three 200-µl portions of ethyl acetate. The extracts were washed with 200 µl of 5 % sodium acetate solution and 200 µl of water and combined. On evaporation, 3.8 mg (4.78×10^6 cpm) of product was obtained. It was separated by preparative TLC with cyclohexane-ethyl acetate (3 : 2) into 1.8 mg of kryptogenin diacetate (**12**) (2.69×10^6 cpm) and 0.6 mg of unreacted **1** (9.26×10^5 cpm).

The **12** was hydrolyzed by refluxing it with 1 ml of methanol and 0.6 ml of 5 % potassium bicarbonate solution for 12 hr. The methanol was removed under a stream of nitrogen, and the aqueous residue was extracted with three 1-ml portions of ethyl acetate. The extracts were washed with two 0.5-ml portions of water, combined, and evaporated. The residue was purified by preparative TLC with dichloromethane-methanol (23 : 2) to give 0.9 mg of chromatographically homogeneous kryptogenin (**13**) (1.60×10^6 cpm).

*Diosgenin-26-^{14}C (**14**).*

Kryptogenin has previously been reduced to Δ^5-cholestene-3β,16β,26-triol-22-one, which cyclizes to diosgenin when treated with acid, either by

hydrogenation with Raney nickel catalyst [11] or by treatment with sodium borohydride [12]. Neither method gives a high yield, however, because of competing side reactions. In our hands, Raney nickel caused considerable reduction of the Δ^5-double bond, while sodium borohydride, a strong base, catalyzes the condensation of the diketone system of kryptogenin to the α,β-unsaturated ketone fesogenin. In addition, both methods cause partial reduction of the 22-keto group. In theory, the latter reaction could be suppressed by operating under acidic conditions, since the desired product would then be converted to diosgenin as it was formed. Sodium borohydride is immediately destroyed by acid, however, and Raney nickel hydrogenation was ineffective in acetic acid. The problem was finally solved by using trimethylamine-borane, one of the few reducing agents for ketones which is stable to acid.

Trimethylamine-borane was prepared by the method of Nöth and Beyer [13]. Kryptogenin-26-^{14}C (2.9×10^5 cpm) was treated with 0.5 mg of trimethylamine-borane in 200 µl of acetic acid at 25° C for 16 hr. The solution was evaporated to dryness in vacuum, and the residue was subjected to preparative TLC with dichloromethane-methanol (47 : 3). Elution of the zone corresponding to diosgenin gave 0.2 mg (2.7×10^5 cpm). An aliquot of this material was acetylated and examined by continuous development TLC [14] with hexane-dichloromethane (1 : 4) for 7 hr. A scan* of the chromatogram showed a single radioactive peak corresponding to diosgenin acetate. No significant radioactivity was observed corresponding to yamogenin acetate, the C-25 epimer of diosgenin acetate. This demonstrates that no racemization of the asymmetric center at C-25 occurred during the synthesis.

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* Strip Scanner I, Atomic Accessories, Valley Stream, New York.