SYNTHESIS OF ^{195}Au, ^{32}P AND ^{35}S-LABELLED AURANOFIN,‡ A NOVEL ORALLY EFFECTIVE GOLD ANTIARTHRITIC DRUG

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SUMMARY

Auranofin (1), a phosphine gold(I) thiolate antiarthritic agent effective on oral administration, was labelled separately with ^{195}Au, ^{32}P and ^{35}S. ^{195}Au-labelled auranofin 19 was made from $H^{195}AuCl_4 \cdot 3H_2O$ via the intermediate chloro(triethylphosphine)gold-^{195}Au (18). Auranofin-^{35}S (23) was prepared using thiourea-^{35}S. The synthetic routes for 19 and 23 were similar to those used for unlabelled 1. However, auranofin-^{32}P (28) was made by the novel, direct coupling of the 1:1 complex (2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranosato-S)gold (27) with $^{32}P(C_2H_5)_3$ (26) synthesized from $^{32}PCl_3$ (24). The labelled compounds were used in pharmacokinetic, tissue distribution and metabolism studies.

Keywords: Auranofin, Antiarthritic, Gold(I) Thiolate, Gold-195, Phosphorous-32, Sulfur-35.

INTRODUCTION

Auranofin (1) ('Ridaura', Smith Kline and French Laboratories), a phosphine coordinated gold(I) thiolate has been found useful in treating rheumatoid arthritis (RA) when administered orally.[1,2] This compound, whose assigned stereochemistry has been confirmed by X-ray crystallographic studies,[3] is presently undergoing extensive clinical investigation and differs in a number of ways from the traditional chrysotherapeutic agents gold sodium thiomalate (2) (Myochrysine) and gold thioglucose (3) (Solganal). Although

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‡ C.A. name: (2,3,4,6-Tetra-O-acetyl-1-β-D-glucopyranosato-S)-(triethylphosphine)gold.

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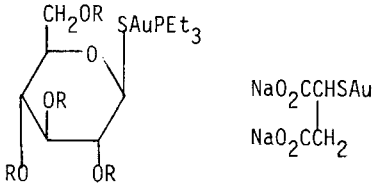
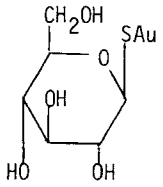
water soluble, 2 and 3 must be administered by injection in order to be effective. Compared with 2, orally active auranofin (1), a lipophilic crystalline material, appears to have a more rapid onset of action in RA patients[4] and has a longer half-life[5]. In addition, auranofin's pharmacological profile shows marked differences compared with 2 and 3[6].



1 R= $CH_3C(=O)-$

2



3

Atomic absorption spectrophotometric analysis of the gold content of excreta from twelve patients receiving 1 showed 95% of the recovered gold to be in the feces and 5% in the urine. This contrasts with 2 where 70% of the recovered gold was found in the urine and 30% in the feces.[5] Radioactive forms of 1 were required to study its absorption and metabolism. Described here are the syntheses of auranofin (1) labelled separately with the inorganic radioactive isotopes ^{195}Au, ^{32}P and ^{35}S. These materials have been employed in pharmacokinetic and tissue distribution studies of 1.[7]

RESULTS AND DISCUSSION

Because of its convergent nature, the previously described[8] preparation of unlabelled 1 (Scheme I) provided the basis for the present radioactive synthesis of both auranofin-^{195}Au (19) (Scheme II) and auranofin-^{35}S (23) (Scheme III). An alternate procedure was chosen for the synthesis of auranofin-^{32}P (28) (Scheme IV). Chloro(triethylphosphine)gold (8), a key intermediate in the synthesis 23 as well as 1, is made by reduction of chloroauric acid (4) to give the water-soluble chloro(thiodiethanol)gold(I) (6). Reaction of 6, formed in situ, with triethylphosphine (7) gives 8 which separates from the aqueous solution as a solid. The carbohydrate moiety is made in standard fashion[9] by conversion of D-glucose pentaacetate (11) to acetobromoglucose (12) followed by treatment with thiourea to give the anomeric salt 14 required for the synthesis of 1, 19 and 28. Alkaline hydrolysis of 14 to generate in situ the potassium salt 15 followed by addition of 8 and coupling yields 1. Similarly, radiolabled gold was incorporated into 1, i.e. 19 in the same fashion by coupling 15 with chloro(triethylphosphine) gold-^{195}Au (18). The latter compound was made by reduction of auric-^{195}Au trichloride trihydrate (16) in 0.1N HCl followed by addition of triethylphosphine (7).

Incorporation of ^{35}S was achieved by reacting thiourea-^{35}S (20) with acetobromoglucose (12) to give the isothiouronium salt 21 in 58% yield. Alkaline hydrolysis of 21 followed by addition of 8 and coupling gave auranofin-^{35}S (23) in overall yield of 19% based on thiourea-^{35}S (20).

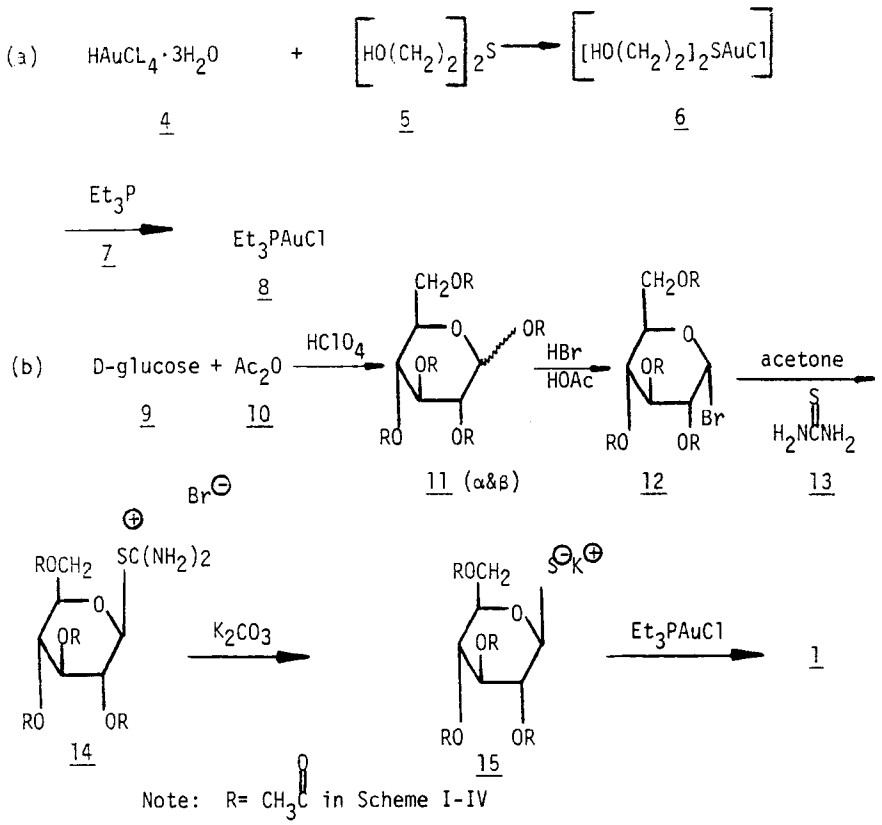
Prior to preparing auranofin-^{32}P (28) two alternate routes were considered. The first envisioned synthesis of chloro(triethylphosphine-^{32}P)-gold (29) and coupling in the usual manner with 15 to give the product 28. A second was direct reaction of triethylphosphine-^{32}P (26) with the 1:1 complex

(2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranosato-S)gold (27). This approach had been reported previously to give unlabelled auranofin (1) in high yield.[10] Although both routes require the preparation of triethylphosphine-^{32}P, the latter would give the final product, 28, with fewer manipulations of radiolabelled phosphorous compounds by eliminating the need for intermediate 29. This is particularly important because of the volatility (b.p. 126-128°C) and pyrophoric nature of 7. The high energy β-emission of ^{32}P together with its relatively short half-life (14.3 days) underscores the need for reduced handling. Based on these considerations, we elected to pursue the second route as shown in Scheme IV.

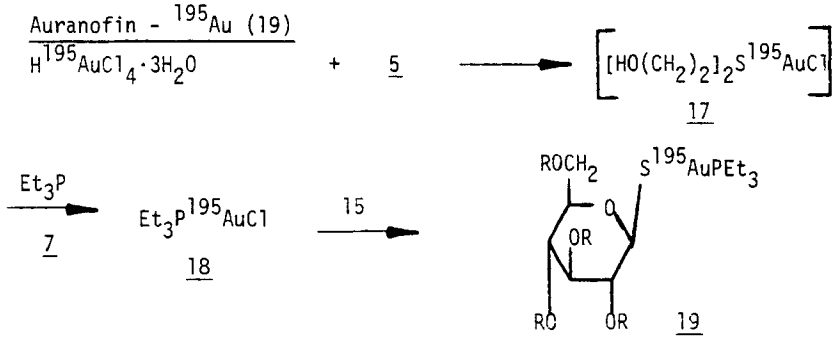
The monocoordinate gold(I) thiolate 27 was made by reacting 6 with the potassium salt 15 prepared *in situ* (see Experimental). The precipitate which formed was collected, washed with water and dried *in vacuo* to give 27 in 96% yield. This material may be recrystallized (1X) from isopropanol-ether (m.p. 146-148°C) Since 1:1 gold(I) thiolates are oligomeric[2] and additional manipulation may increase the degree of polymerization rendering them insoluble, 27 was used directly without further purification.

Triethylphosphine-^{32}P (26), made by addition of $^{32}PCl_3$ (24) to ethyllithium (25) in hexane, was added to an acetonitrile solution of 27. Workup, which included chromatography on Florisil, crystallization and final purification by HPLC gave 28 in 14.7% yield based on 27.

Scheme I

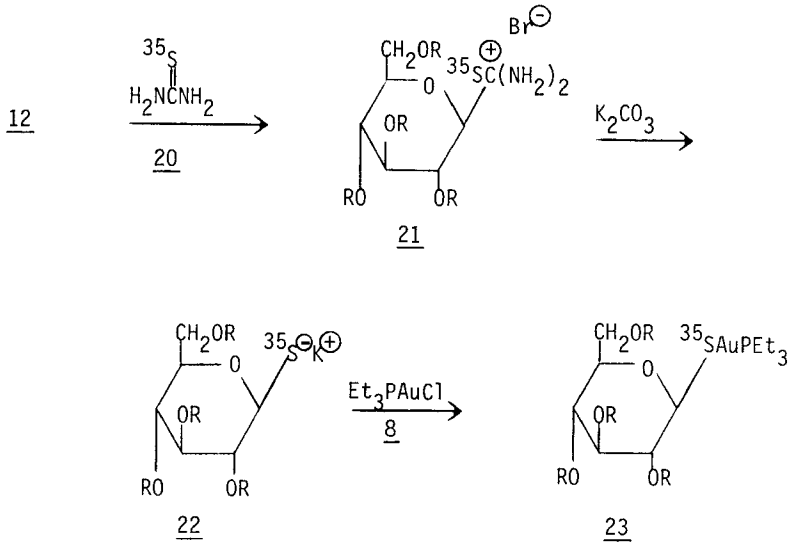


Scheme II



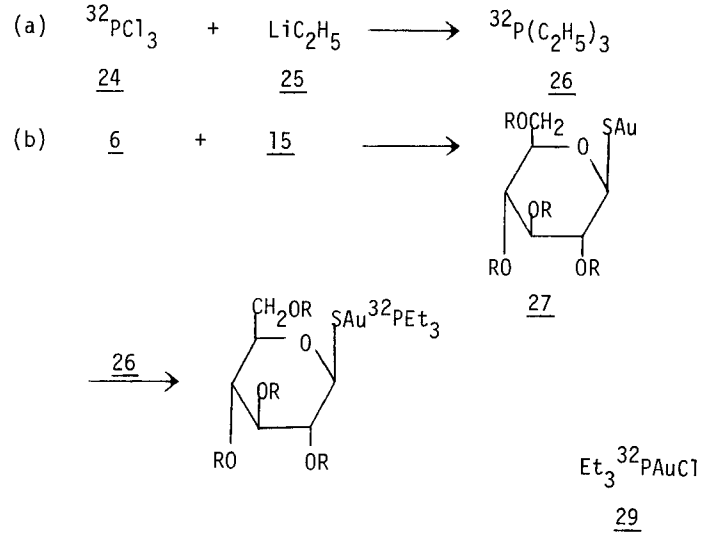
Scheme III

Auranofin -^{35}S (23)



Scheme IV

Auranofin -^{32}P (28)



EXPERIMENTAL

Auric-^{195}Au trichloride trihydrate (16) (20 mCi-carrier free) in 1.0 ml of 0.5 N HCl (chloroauric acid) was purchased from New England Nuclear, Boston, MA. and unlabelled chloroauric acid (4) from Engelhard Industries Inc. Phosphorous -^{32}P trichloride (24) was purchased from Amersham Corporation, Arlington Heights Illinois. Sulfur-35 labelled (2,3,4,6-tetra-O-acetyl-β-D-glucopyranosato-S)-(triethylophosphine)gold (23) was synthesized by New England Nuclear by a procedure that was developed in our laboratory and described in this paper. Chloro (triethylphosphine)gold (8) was prepared as reported[8] as were the various sugar derivatives (11, 12, 14, 15).[8,9]

Distilled water is used in all instances where water is indicated. Temperatures reported are centigrade. Radiochemical purity was determined by TLC radioscan using a Berthold scanner model LB2760. Analtech Silica Gel GF TLC plates were used; UV light at 254 nm was used to visualize the separated components. The specific activities were determined by liquid scintillation counting using an internal standard method with an Amersham Searle Mark III liquid scintillation counter model 6880. Preparative liquid chromatography (28) was carried out on a Bondapak C_{18}/Porasil B (37-75 micron) column (7mm x 61 cm) in conjunction with an Altex pump and LDC Spectromonitor III UV detector.

Chloro(triethylphosphine)gold-^{195}Au(18)

A solution of 20 mCi of carrier-free auric-^{195}Au trichloride trihydrate in 0.5 ml. of 0.1 N HCl was diluted with a solution of 0.79 g (2.0 mmol) of unlabelled chloroauric acid in 1.0 ml of water. The resulting yellow solution was cooled to 5°. A solution of 0.40 g (4.0 mmol) of 2,2'-thiodiethanol in 2.0 ml of isopropyl alcohol was added slowly and the reaction was stirred at 5° for 10 min. A solution of 0.26 g (2.2 mmol) of triethylphosphine (7) in

1.5 ml of isopropyl alcohol was added slowly causing a precipitate to form. Stirring was continued at 5° for 1 hr, the precipitate was then filtered and washed with a minimum of cold water. The white solid was dried at ambient temperature in vacuo to give 0.43 g (60.7% yield) of 18. Radiochemical purity was 97.8% (ethylacetate-hexane 1:1 v/v).

Purification of Chloro(triethylphosphine)gold-^{195}Au(18)

A solution of 0.43 g (1.21 mmol) of chloro(triethylphosphine)gold-^{195}Au (18) dissolved in 1.0 ml of dichloromethane was placed on a pre-packed silica gel column (60-200 mesh). The column was eluted with dichloromethane and monitored radiolytically. The radioactive fractions were combined and concentrated in vacuo yielding a clear oil that solidified to a white solid on standing. After drying at ambient temperature in vacuo, the solid weighed 0.42 g (99% recovery)

(2,3,4,6,-Tetra-O-acetyl-1-thio-β-D-glucopyranosato-S)(triethylphosphine)-gold-^{195}Au (19)

A solution of 0.62 g (1.27 mmol) of 1-thio-2,3,4,6-tetraacetate-1-carbamimidate-β-D-glucopyranose monohydrobromide (14)[9] in 1.6 ml methyl alcohol and 1.4 ml of water was cooled to 5°. After 10 min, a solution of 0.233 g (1.69 mmol) of potassium carbonate in 1.9 ml of water was added slowly causing a white solid to precipitate. The reaction was stirred at 5° for 10 min then removed from the cooling bath. A solution of 0.42 g (1.21 mmol) of chloro-(triethylphosphine)gold-^{195}Au (18) in 3.0 ml of methyl alcohol was added slowly. The reaction was allowed to warm to ambient temperature over 1 hr during which time the precipitate redissolved. The reaction solution was filtered through a 2 mm cellulose filter cake to remove particulate material. The cake was washed with 1.0 ml of methyl alcohol and the washing was combined with the

filtrate. The filtrate was diluted with 1.5 ml of water, seeded and cooled to 5° (1 hr). The crystalline solid was collected and washed with 2.0 ml of a cold methyl alcohol: water mixture (1:1 v/v). The white solid was dried at ambient temperature in vacuo to give 0.35 g (43% yield) of 19. Radiochemical purity was 97.4%; specific activity 8.80 mCi/mmol; (toluene-ethyl acetate-hexane-ammonium hydroxide 60:40:10:0.2 v/v). This material was recrystallized by dissolving in a minimum of methyl alcohol at ambient temperature, then adding water until the solution began to turn cloudy. The solution was seeded and cooled. The resulting precipitate was collected and dried giving an 80% recovery.

1-Thio-^{35}S-2,3,4,6-tetraacetate-1-carbamimidate-β-D-glucopyranose monohydrobromide (21)

To a solution of 1.3 ml of a acetic acid saturated with hydrogen bromide gas cooled to 15-20° was added 0.672 g (1.72 mmol) of D-glucose pentaacetate (11). The reaction was stirred at ambient temperature for 1 hr. The reaction was poured into 10 ml of ice water causing a white solid, acetobromoglucose (12), to precipitate (this intermediate is unstable on standing). The solid was collected, dissolved in chloroform and washed with water followed by a solution of saturated sodium bicarbonate. The chloroform solution was dried over magnesium sulfate and concentrated in vacuo to give 0.65 g (91% yield) of 12 as a gum. A solution of 0.22 g (0.54 mmol) of 12 in 0.7 ml of acetone was treated with 0.072 g (0.95 mmol) of thiourea-^{35}S (57.2 mCi with a specific activity of 60.4 mCi/mmol). The solution was refluxed for 1.0 hr and cooled to 5° causing a white solid to precipitate. The solid was filtered, washed with cold acetone, and dried in vacuo at ambient temperature to give 0.153 g (58% yield) of 21. An additional 0.014 g was obtained from the mother liquors.

(2,3,4,6-Tetra-O-acetyl-1-thio-β-D-glucopyranosato-S)(triethylphosphine)gold -^{35}S (23)

A solution of 0.167 g (0.34 mmol) of 1-thio-2,3,4,6-tetraacetate-1-carbamimidate-β-D-glucopyranose-^{35}S monohydrobromide (21) in 0.4 ml methyl alcohol and 0.3 ml of water was cooled to 5°. After 10 min 1.17 g (8.48 mmol) of potassium carbonate in 10 ml of water was added slowly causing a white solid to precipitate. The reaction was stirred at 5° for 10 min then removed from the cooling bath. A slurry of 0.115 g (0.33 mmol) of chloro(triethylphosphine)-gold (8) in 0.7 ml of methyl alcohol was added slowly. The reaction was allowed to warm to ambient temperature over 45 min during which time the precipitate redissolved. The reaction was cooled to 0°. After 10 min a white solid precipitated. The mixture was stirred for 30 min and then filtered. The wet crystals were dissolved in 1.6 ml of methyl alcohol and filtered. The filter funnel was washed with 0.7 ml of methyl alcohol. Water (3.0 ml) was added to the filtrate and the filtrate was cooled to 0° causing a solid to precipitate. The crystals were isolated by filtration and dried in a dessicator over P_2O_5. The dried white solid weighed 0.136 g (59% yield). The overall radiochemical yield from thiourea-^{35}S was 19%. The radiochemical purity was 98.0% (toluene-ethyl acetate-hexane-ammonium hydroxide 60:40:10:0.2 v/v); specific activity was 43.7 mCi/mmol.

(2,3,4,6-Tetra-O-acetyl-1-thio-β-D-glucopyranosato-S)gold (27)

To a solution of 2.75 g (22.54 mmol) of 2,2'-thiodiethanol in 4.0 ml of isopropyl alcohol at 5° was added a solution of 2.47 g (6.26 mmol) of chloroauric acid in 3.0 ml of water while maintaining the temperature at 5-10°. The addition was accompanied by the precipitation of a red solid which redissolved immediately yielding a colorless solution. After stirring the reaction

at 5° for 12 min a solution of 1.68 g (12.2 mmol) of potassium carbonate and 2.4 g (6.59 mmol) of 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranose[8] dissolved in a mixture of 12.0 ml of water and 5.0 ml of ethyl alcohol was added slowly. The addition was accompanied by foaming and the precipitation of a white solid. The reaction was stirred at 5° for 0.5 hr. The solid was filtered, washed thoroughly with water and dried at ambient temperature in vacuo giving 3.39 g (96% yield) of 27.

(2,3,4,6-Tetra-O-acetyl-1-thio- β -D-glucopyranosato-S)(triethylphosphine)gold-^{32}P (28)

A solution of ethyllithium in benzene (27.5 ml, 34.4 mmol) under an argon atmosphere was diluted with 8 ml of toluene and then cooled in a dry ice-acetone bath. To this solution was added 0.76 g (5.5 mmol) of phosphorous-^{32}P trichloride (131 mCi). The reaction was warmed slowly to 50° for 30 min, cooled in an ice bath and quenched slowly with 4.5 ml of 10% sodium hydroxide. The organic layer was separated, dried over magnesium sulfate and added to a solution of 2.45 g (4.4 mmol) of 27 in 7.0 ml of acetonitrile. The reaction was stirred at ambient temperature for 15 min. An excess of 0.71 g (6.5 mmol) of unlabelled triethylphosphine (7) was added to ensure complete reaction. The reaction mixture was stirred at ambient temperature for an additional 15 min, then concentrated in vacuo yielding a yellow oil. The oil was eluted through a Florisil column (100-200 mesh), with dichloromethane. The eluent upon concentration in vacuo gave an oil which was dissolved in 6.0 ml of methyl alcohol, diluted with water to the cloud point (6.0 ml), then cooled. The product separated as a gum; it was removed and redissolved in 8.0 ml of methyl alcohol. An additional 0.5 g (0.737 mmol) of unlabelled (2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranosato-S)(triethylphosphine)gold (1) was dissolved in the methyl alcohol, followed by enough water to reach the cloud point (11.0 ml).

The solution was cooled in an ice bath and seeded. A white solid separated, it was collected, washed with a methyl alcohol-water mixture (1:1 v/v) and dried at ambient temperature in vacuo to give 0.44 g (2% radiochemical yield of 28). Radiochemical purity was 99.2% (toluene-chloroform-methyl alcohol 60:40:10 v/v) specific activity was 0.46 mCi/mmol.

Purification of (2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranosato-S)(triethylphosphine)gold-^{32}P (28)

Purification of 0.44 g (0.73 mmol) of 28 was achieved by preparative liquid chromatography with methyl alcohol-0.01M NaH_2PO_4 (65:35) as the mobile phase. Ultra-violet light (254 nm) was used for detection. The main fraction was concentrated yielding an oil which was redissolved in 11.0 ml of a methyl alcohol-water (7:4 v/v) solution. Water was added until the cloud point was reached (10 ml); the solution was seeded and cooled to 5°. The solid was filtered, washed with a small amount of a methyl alcohol-water mixture (1:1 v/v) and dried at ambient temperature in vacuo yielding 0.32 g (64% recovery). Radiochemical purity of 28 was 99.2% (toluene-chloroform- methyl alcohol 60:40:10 v/v); specific activity was 0.46 mCi/mmol.

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