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Oral Gold. Antiarthritic Properties of Alkylphosphinegold Coordination Complexes

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Preparation and biological structure-function relationships of a series of trialkylphosphinegold complexes are reported. Several examples show antiarthritic properties when administered orally to adjuvant arthritic rats. Triethylphosphinegold complexes appeared most effective.

Development of specific therapeutic agents for arthritic diseases is complicated by the biological diversity of this large group of related diseases.[1] Much of the medication used today acts symptomatically to suppress pain and acute inflammation but rarely affects ongoing disease processes.[2] It was our objective to develop improved therapy specific for rheumatoid arthritis, and we directed our attention to the benefit obtained in this disease with long-term chrysotherapy. Monovalent gold salts often bring about permanent remission of the active disease in both the adult and juvenile forms.[3] Radiologic evidence indicates that they slow progression of joint involvement,[4] and other data suggest that they modify several biological systems associated with the pathogenesis of rheumatoid arthritis:[5,6] lysosomal enzymes,[7] sulfhydryl interchange,[8] collagen stabilization.[9]

This paper reports on the preparation and comparative antiarthritic properties of a group of phosphinegold coordination compounds which show the unique property of being absorbed on oral administration, whereas present day chrysotherapeutics must be administered parenterally for therapeutic effect.

Coordination of monovalent Au with ligands of the phosphine type is known to stabilize the reduced valence state of the metal and result in nonionic complexes, soluble in organic solvents.[10,11] The nature of the coordinating agent also influences the distribution pattern of Au in animal tissues.[12] Table I lists the compds that were prepd for study. All show a high degree of lipid solubility[10,11,†] in contrast to H_2O-soluble Au salts, and oral administration of many prevented the development of adjuvant-induced arthritis in experimental animals (Table II). Plasma Au levels confirmed that these compds, in contrast to presently used Au salts, were absorbed efficiently when administered orally. Their value as improved therapy in the treatment of rheumatoid arthritis is being studied.

Chemistry. Chloro(trialkylphosphine)gold complexes (I) were prepd in a direct manner, as reported previously,[13] by allowing a soln of trialkylphosphine to react with AuCl in a polar solvent. AuCl was prepd freshly *in situ* by reducing an aqueous soln of $HAuCl_4$ with thiodiglycol. The resulting soln was reacted immediately with a cold EtOH soln of the trialkylphosphine.

Treatment of aqueous solns of sodium thioglucose with I gave thioglucose complexes (II) as hygroscopic products.

Careful hydrolysis of 2-(tetra-*O*-acetyl-β-D-glucopyranosyl)-thiopseudourea hydrobromide with K_2CO_3 gave the salt of the acylated thiosugar which was allowed to react further with I to form [(tetra-*O*-acetyl-β-D-glucopyranosyl)thio]-(trialkylphosphine)golds (III).

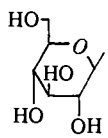
Treatment of the *O*- and *S*-β-mercaptoethyl glycosides of acetylglucose with NaOMe, followed by reaction with I, resulted in acetolysis and formation of trialkylphosphinegold complexes of the mercaptoethyl glycosides (IV).

$R_3P + AuCl \longrightarrow R_3PAuCl$

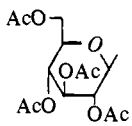
I, R = Me, Et, *i*-Pr, *n*-Bu

$I + R'S^- \longrightarrow R_3PAuSR'$

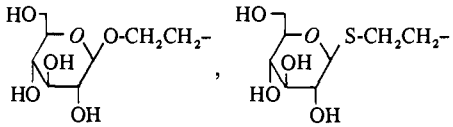
II, R = Me, Et, *i*-Pr, *n*-Bu; R' =



III, R = Et, *n*-Bu; R' =



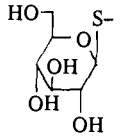
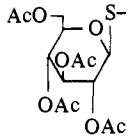
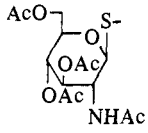
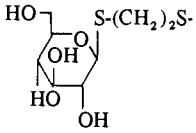
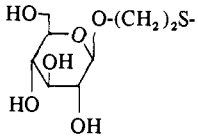
IV, R = Et; R' =



Biology. Structure-Function Relationships. Oral administration of soluble Au salts is ineffective in protecting rats from the development of adjuvant arthritis produced by administration of complete Freunds adjuvant;[14,15] no significant Au levels were detectable in the sera of those experimental animals. All compds reported here in which Au was in the form of a phosphine complex gave demonstrable serum Au levels after oral administration to rats, and the

†D. E. Guttman, Analytical and Physical Chemistry Laboratories, Smith Kline and French Laboratories, Philadelphia, Pa., private communication. Solubility of 1 in $CHCl_3$ > 100 mg/ml; in Et_2O 7.5 mg/ml, cyclohexane 0.4 mg/ml; partition coefficient H_2O–$CHCl_3$ 0.042, H_2O–Et_2O 0.11, H_2O–cyclohexane 0.72.

Table I. Trialkylphosphinegold Coordination Complexes (R_3PAuR')

No.	R	R'	Formula[a]	Mp, °C	Crystn solvent	Yield, %	$\alpha^{25}D$
1	Et	Cl	$C_6H_{15}AuClP$	85–86[b]	EtOH–H_2O	90	
2	Me	Cl	C_3H_9AuClP	228–229	EtOH	95	
3	*i*-Pr	Cl	$C_9H_{21}AuClP$	184–186	EtOH–CH_2Cl_2	88	
4	*n*-Bu	Cl	$C_{12}H_{27}AuClP$	*c*		74	
5	Me		$C_9H_{20}AuO_5PS$	130–132	EtOH	81	+8.6[d]
6	Et		$C_{12}H_{26}AuO_5PS$	*e*		64	+8.7[f]
7	*i*-Pr		$C_{15}H_{32}AuO_5PS$	*g*		63.6	+7.6[h]
8	*n*-Bu		$C_{18}H_{38}AuO_5PS$	*i*		93	+7.1[j]
9	Et		$C_{20}H_{34}AuO_9PS$	110–111		90	−55.3[k]
10	*n*-Bu		$C_{26}H_{46}AuO_9PS$	*l*		89	−39.8[m]
11	*n*-Bu		$C_{26}H_{47}AuNO_8PS$	*n*		80	
12	Et		$C_{14}H_{30}AuO_5PS$	*o*		56	−20.7[p]
13	Et		$C_{14}H_{30}AuO_6PS$	*q*		61.3	−15.1[r]

[a] All compds were analyzed for C, H, P. [b] Lit.[13] 78. [c] Pale yellow oil after chromatography on silica gel with Et_2O. [d] 1% MeOH. [e] Hygroscopic amorphous solid after chromatography on silica gel with Et_2O–MeOH (1:1). [f] 1% H_2O. [g] Hygroscopic amorphous solid after chromatography on silica gel with C_6H_{12}–Me_2CO (1:3). [h] 0.94% MeOH. [i] Viscous oil after chromatography on silica gel with CH_2Cl_2–Me_2CO (2:1). [j] 1.1% $CHCl_3$. [k] 1% MeOH. [l] Viscous oil after chromatography on silica gel with CH_2Cl_2–Me_2CO (4:1). [m] 1.05% $CHCl_3$. [n] Amorphous solid. [o] Hygroscopic solid after chromatography on silica gel with Me_2CO. [p] 1% MeOH. [q] Hygroscopic solid after chromatography on silica gel with $CHCl_3$–MeOH (9:1). [r] 0.7% MeOH.

degree of arthritis prevention paralleled the magnitude of serum Au concn. Both therapeutic responsiveness and oral absorption properties, as evidenced by serum Au levels, were structure dependent. Although the non-Au phosphine compds studied gave no protection, comparative studies indicated that the nature of the phosphine ligand in the Au coordination complexes played a greater role in bringing about changes in biological activity than did the other group bonded directly to Au. In Table II, class 1, equivalent oral doses of gold in 4 different complexed forms were administered to experimental animals. The alkyl moiety of the halo(trialkylphosphine)gold was varied and the halogen held constant. Serum gold levels, as well as therapeutic effect, were highest with the Et_3P compd (**1**). As the size of the alkyl substituent increased (**3**, **4**), both serum Au levels and therapeutic protection fell. A similar response was produced by the compds in class 2 (Table II) in which the nonphosphine binding groups were thiosugars. Et_3P complexes (**6**, **9**) were more effective both in producing a therapeutic response and developing serum Au levels than were Me (**5**), *i*-Pr (**7**), or *n*-Bu complexes (**8**, **10**, **11**) at equivalent oral doses of Au. The data in class 2 (Table II) also illustrate that the therapeutic responses produced by these compds increase with increasing serum Au levels in the adjuvant rat. Comparison of trialkylphosphinegold complexes in which the phosphine group (R_3P) was constant and the other group varied (Table II) (**1**, **6**, **9**, **12**, **13**) suggests that the nonphosphine group has less effect in controlling absorption characteristics and therapeutic protection than the phosphine. At equivalent dose levels (10 mg/kg) therapeutic responses and sera gold levels correlated favorably, considering biological variation between separate experiments. As noted earlier Pr_3- (**7**) and Bu_3- (**8**, **10**, **11**) containing thiosugars produced poorer responses than the Et_3 congener.

The rat acute oral LD_{50}'s for **1** and **9** were found to be 79.0 (56.9–112.2) mg/kg and 265 (190.6–368.4) mg/kg, respectively. Their antiarthritic activity was not associated with adverse effects on body weight. Oral administration at a dose of 10 mg of gold resulted in a significant reduction of adjuvant-induced body weight loss accompanied with suppression of secondary lesions.

Experimental Section‡

Chloro(trialkylphosphine)golds (1–4). A soln of 10.0 g (0.8 mole) of thiodiglycol in 25 ml of EtOH was mixed with a soln of 15.8 g (0.038 mole) of $AuHCl \cdot 3H_2O$ in 75 ml of H_2O. When the orange soln became colorless, it was cooled to −5° and an equally cold soln, containing 5.0 g (0.043 mole) of alk_3P in 25 ml of EtOH, was added dropwise to the stirred mix. Stirring was continued for

‡ Mp's (uncorr) were detd in a Thomas-Hoover capillary mp apparatus. Optical rotations were measured using a Perkin-Elmer Model 141 polarimeter. Nmr spectra (TMS) were run using either a Varian T-60 or Jeol C-60H spectrometer. All P-contg compds except **2** showed complex CH absorption at 1.6 ppm. **2** gave a simple AX pattern at 1.6 ppm with a J_{HP} = 14 Hz. Subspectral analysis of **1** as an A_3B_2X pattern[16] gave J_{AB} = 7.5, J_{AX} = 18.5, and J_{BX} = −10.2 Hz§ which is in reasonable agreement with published data. Elemental analyses were within ±0.4% of the theoretical values. Serum Au content was obtained by atomic absorption spectrometry[17] using a Perkin-Elmer Model 303 spectrometer. Recrystn solvents and physical data for Au complexes are given in Table I.

§ J. R. Stedman, Temple University, personal communication, 1971.

Table II. Detection of Antiarthritic Properties and Plasma Au Concentration

Compd	Dose, mg/kg per day, calcd as Au	Hind-leg vol % redn from adj control[a]			Au, μg/ml of serum
		Injected leg		Uninjected leg	
		Day 3	Day 16	Day 16	
		Class 1, R_3PAuCl			
1	10	14.38[c]	23.25[d]	36.60[c]	5.38
2	10	9.59[c]	*e*	*e*	1.66
	b	24.60[d]	19.47[d]	32.33[d]	
3	10	14.73[c]	15.79[c]	33.33[c]	3.49
4	10	19.86[d]	*e*	*e*	0.92
		Class 2, R_3PAuSR'			
6	20	27.22[d]	22.44[d]	44.48[d]	6.92
	b	25.79[d]	23.11[d]	42.33[d]	
	10	16.88[d]	*e*	27.20[c]	4.13
	b	39.38[d]	32.82[d]	54.12[d]	
	5	11.25[f]	20.70[c]	34.06[d]	3.53
	b	39.38[d]	32.82[d]	54.12[d]	
9	20	33.81[d]	23.11[d]	55.21[d]	7.89
	b	25.79[d]	23.11[d]	42.33[d]	
	10	30.00[d]	29.74[d]	44.51[d]	4.11
	b	39.38[d]	32.82[d]	54.12[d]	
	5	30.00[d]	*e*	*e*	2.95
	b	39.38[d]	32.82[d]	54.12[d]	
8	20	*e*			*g*
	b	25.79[d]	23.11[d]	42.33[d]	
	10	28.44[d]	*e*	*e*	1.15
	b	39.38[d]	32.82[d]	54.12[d]	
	5	*e*	*e*	*e*	0.92
	b	39.38[d]	32.82[d]	54.12[d]	
10	20	26.07[d]	18.66[c]	36.20[f]	2.04
	b	25.79[d]	23.11[d]	42.33[d]	
	10	19.38[c]	*e*	*e*	1.25
	b	39.38[d]	32.82[d]	54.12[d]	
	5	*e*	*e*	*e*	1.24
	b	39.38[d]	32.82[d]	54.12[d]	
11	20	30.09[d]	15.11[f]	30.37[d]	1.70
	b	25.79[d]	23.11[d]	42.33[d]	
	10	21.88[d]	*e*	*e*	1.08
	b	39.38[d]	32.82[d]	54.12[d]	
	5	26.25[d]	*e*	*e*	1.15
	b	39.38[d]	32.82[d]	54.12[d]	
5	10	14.45[c]	15.96[f]	*e*	2.3
	b	30.38[d]	37.32[d]	37.10[f]	
7	10	18.24[d]	15.35[f]	15.76[f]	*h*
	b	35.50[d]	41.93[d]	47.23[d]	
	5	*e*	*e*	*e*	1.63
	b	23.47[d]	36.75[d]	38.60[f]	
		Class 3			
12	10	9.59[f]	27.19[d]	33.99[c]	4.8
13	10	17.47[d]	30.04[d]	44.44[d]	5.7

[a] % redn from adj control = (hind-leg vol of untreated adj control rat − hind-leg vol of treated control rat)/hind-leg vol of untreated adj control rat. Untreated non-adj-control hind-leg vol approximates a 50% redn from that of adj control rat. [b] Prednisolone treated, 20 mg/kg per day. [c] $0.001 < P < 0.01$. [d] $p < 0.001$. [e] No significant redn in paw vol. [f] $0.01 < p < 0.05$. [g] All animals died by day 16. [h] Au content not detd.

0.5 hr, then the sepd solid was removed. Upon concn of the filtrate to 30 ml, a second solid sepd. The combined material was washed with cold H_2O–EtOH (2:1) and recrystd.

(Trialkylphosphine)[(β-D-glucopyranosyl)thio]golds (5–8). A cold (−10°) soln of 3.08 g (0.01 mole) of **2** in 30 ml of Me_2CO was added dropwise to an equally cold, stirred soln of sodium thioglucose dihydrate (2.54 g, 0.01 mole) in 25 ml of H_2O. Stirring with cooling was continued for 1 hr. The solid sepn was removed by filtration, and the filtrate was concd under reduced pressure to yield a second crop of solid. The combined solid was washed with H_2O–Me_2CO (2:1) and then H_2O, dried, and purified by silica gel chromatography

[(Tetra-*O*-acetyl-β-D-glucopyranosyl)thio](trialkylphosphine)golds (9–10). A cold soln of 1.66 g (0.012 mole) of K_2CO_3 in 20 ml of H_2O was added to a soln of 5.3 g (0.011 mole) of 2-(tetra-*O*-acetylglucopyranosyl)-2-thiopseudourea hydrobromide[18] in 30 ml of H_2O at −10°. A cold soln of 3.86 g (0.011 mole) of 1 in 30 ml of EtOH contg a few drops of CH_2Cl_2 was added to the mixt before hydrolysis of thiouronium salt was complete. After complete addn, the mixt was stirred in the cold for an addnl 0.5 hr. The sepd solid was removed, washed with H_2O–EtOH and H_2O, and dried under reduced pressure.

[(2-Acetamidotri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)thio](tributylphosphine)gold (11). 2-(2-Acetamidotri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2-thiopseudourea hydrochloride[19] was reacted in the same manner as with **9**.

(Triethylphosphine){[2-(β-D-glucopyranosylthio)ethyl]thio}gold (12). A soln of 1.62 g (0.03 mole) of NaOMe in 25 ml of MeOH was added to an ice-cooled mixt of 4.66 g (0.01 mole) of **14** in 75 ml of MeOH under N_2. When a complete soln resulted, a cold soln of 3.5 g (0.01 mole) of **1** in 25 ml of MeOH was added dropwise. Stirring in the cold was continued for 0.5 hr before the entire mixt was concd under reduced pressure. The residue was purified by chromatography on silica gel with Me_2CO development.

(Triethylphosphine){[2-(β-D-glucopyranosyloxy)ethyl]thio}gold (13). **15** was treated in the same manner as **12**.

2-Acetylthioethyl Tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (14). A mixt of 3.99 g (0.034 mole) of KSAc (prepd *in situ* from KOH and AcSH) and 16.47 g (0.035 mole) of 2-bromoethyl tetra-*O*-acetyl-1-

thio-β-D-glucopyranoside[20] in 125 ml of EtOH was stirred at reflux temp for 1.5 hr. Afterwards, the mixt was concd under reduced pressure and the residue partitioned between H_2O and $CHCl_3$. The organic exts were dried (Na_2SO_4), filtered, and concd *in vacuo*, and the residue was recrystd from MeOH to give 14.2 g (87%) of solid: mp 110–112°; [α]D ($CHCl_3$) −33.2° (*c* 1.246). *Anal.* ($C_{18}H_{26}O_{10}S_2$) C, H, S.

2-Acetylthioethyl Tetra-*O*-acetyl-β-D-glucopyranoside (15). An EtOH soln of 5.85 g (0.014 mole) of 2-chloroethyl tetra-*O*-acetyl-β-D-glucopyranoside[21] was allowed to react with KSAc as in 14. The residue was recrystd from MeOH to give 4.6 g (72%): mp 80–81°; [α]D ($CHCl_3$) −3.8° (*c* 1.016). *Anal.* ($C_{18}H_{26}O_{11}S$) C, H, S.

Antiarthritic Assay Method.[14] Adjuvant arthritis was produced by a single intradermal injection of 0.75 mg of *Mycobacterium butyricum* suspended in white paraffin oil (light N.F.) into the left hind-paw footpad. The injected leg becomes inflamed (increased vol) and reaches max size within 3–5 days (primary lesion). The animals exhibit a decrease in body wt gain during this initial period. Adjuvant arthritis (secondary lesion) occurs after a delay of approx 10 days and is characterized by inflammation of the noninjected sites (right hind leg), decrease in body wt gain, and further increases in the vol of the injected hind leg. Test compds were administered daily, beginning on the day of adjuvant injection for 17 days exclusive of days 4, 5, 11, and 12. Drug activity on the primary (left leg day 3) and secondary (both legs day 16) lesions was detd by comparing leg vols of the treated group with a control arthritis (vehicle) group. Hind-leg vols were measured by immersing the leg into a Hg reservoir and recording the subsequent Hg displacement. A compd was considered to have antiarthritic activity if it produced a statistically significant ($p < 0.05$) decrease in the inflamed hind-leg volumes when compared with arthritic controls. The level of significant difference between treated groups and control groups was determined by the Student's *t* test.

Acute Single Dose Lethality. Male Charles River rats weighing between 160 and 220 g were divided randomly into groups of ten rats each. The groups were treated orally with test compd. After dosing, all animals were obsd for acute deaths on day 1 and delayed deaths over a 10-day period. All LD_{50}'s and their confidence limits were calcd using the Litchfield–Wilcoxon method.[22]

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New Substrates for a Pancreatic Exocrine Function Test

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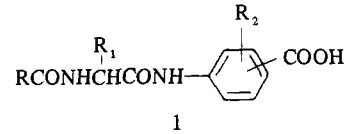
Three-unit compounds, such as 4-(*N*-benzoyl-L-tyrosyl)aminobenzoic acid which, in general, comprise a sensitive peptide linkage to tracer aminobenzoic acids, were prepared and shown in rat tests to possess considerable discrimination for *in vivo* chymotrypsin activity when ingested. They may, therefore, be useful in the clinical detection of pancreatic exocrine insufficiency. Thirteen of these compounds are listed in Table I. The peptide linkage was formed by the unique reaction of mixed anhydride of the appropriate acylated α-amino acid with the free aminobenzoic acid, with toluenesulfonic acid catalyst. *In vitro* half-time values were determined for the hydrolysis of these compounds in the presence of chymotrypsin and are compared with *in vivo* data. Ortho substitution in the aminobenzoic acid portion strongly affected both optical activity and hydrolysis rate.

Reliable clinical measurement of pancreatic exocrine function must be carried out on duodenal aspirates and is therefore difficult to conduct on a routine basis. As part of an attempt to devise a simpler test, we have prepared new compounds containing a peptide bond, which, when ingested, would be split specifically by the pancreatic enzyme, chymotrypsin, to yield a nontoxic tracer which could be recovered in the urine. This paper reports the chemistry, some *in vitro* rate data, and correlation of the latter with *in vivo* data on rats. Tests in three animal species have been reported for compound 1.[1]



1

We selected *p*-aminobenzoic acid (PABA) and, more generally, the aminobenzoic acids (ABA's) as the tracers, be-