Proton, Carbon-13, and Phosphorus-31 Nuclear Magnetic Resonance Studies of (2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-glucopyranosato-*S*)-(triethylphosphine)gold (Auranofin), a Novel Antiarthritic Agent

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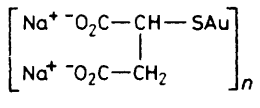
The ^1H and ^{13}C high-field n.m.r. spectra of auranofin in a variety of aqueous and non-aqueous solvents have been analysed and all peaks unambiguously assigned except those for the acetyl groups. Specific solvation and co-ordination shifts are discussed. The conformation in solution appears to resemble that in the crystal, with the glucopyranose ring in a chair form. Phosphorus-31 n.m.r. studies suggest that auranofin is relatively stable at pH 7 and 4, but not at pH 1.

Rheumatoid arthritis, a progressive disease involving destruction of joint cartilage tissue, has been controlled in many cases over the past fifty years by the injectable gold(I) thiolates (1) (Myocrisin) and (2) (Solganol).[1-3] An orally administered form of gold, auranofin [(2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranosato-*S*)(triethylphosphine)gold (3) ('Ridaura,' Smith Kline and French Laboratories)] is at present undergoing extensive clinical trial for the treatment of rheumatoid arthritis. It is efficacious and well tolerated, and exhibits therapeutic properties superior to the traditional chrysotherapeutic agents.[4-6] *X*-Ray crystallography shows that in structure (3) the gold is co-ordinated to both a sulphur and a phosphine ligand in linear fashion (SAuP 173.6°).[7] Because of the simplicity of the ^{31}P n.m.r. spectrum and the sensitivity of ^{31}P chemical shifts to substitution *trans* to the phosphorus nuclei, ^{31}P n.m.r. has served as a convenient probe for monitoring the fate of phosphine-co-ordinated gold drugs including (3) in their interaction with biological systems.[8,9] Conceptually, other nuclei of (3) could also be employed as probes in biological studies if their chemical shift assignments were known. Using a very high-field n.m.r. spectrometer and homo- and hetero-nuclear decoupling techniques together with various solvents, we have been able to assign unequivocally the peaks in both the ^1H and ^{13}C spectra of (3). These results are described here together with the ^{31}P n.m.r. data.



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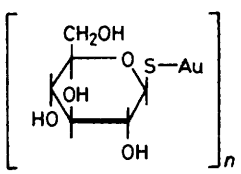

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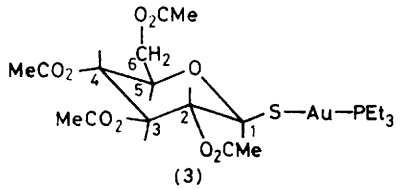

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Results and Discussion

^1H *N.M.R. Spectra.*—The high-field (400 MHz) ^1H n.m.r. spectra of the glucopyranose ring of auranofin in CD_3OD, $(CD_3)_2SO$, and $CDCl_3$ are shown in Figure 1. Chemical shifts and selected coupling constants are listed in Table 1. The latter were derived by computer simulation of spectra where necessary. The ring protons of auranofin gave complex second-order ^1H spectra at low fields (60—220 MHz); even at 400 MHz [Figure 1(a)] in $CDCl_3$, the ^1H n.m.r. spectrum cannot be analysed simply, mainly owing to long-range virtual coupling.[10] Fortunately the chemical shifts show remarkable and specific solvation shifts, and analysis is aided further by the utilization of very high-field spectrometers. Thus, the 400-MHz spectrum from a methanol solution, for example, is nearly first order [Figure 1(c)]. Such solvation shifts are not unique to auranofin. They were noted previously by Lemieux and Stevens[10] and Holland *et al.*[11] during their studies of 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose itself.

The resonances of C(2)H, C(4)H, and C(6)H_A in particular show large solvation shifts (*ca.* 0.2 p.p.m.) to low field on transference of auranofin from $(CD_3)_2SO$ to CD_3OD (Table 2). These protons are all on the ' top side ' (β) of the molecule. The same trend is observed from $(CD_3)_2SO$ to D_2O, and slightly magnified (0.3—0.4 p.p.m.), suggesting that the solvent shifts involve H-bonding between the solvent and the oxygen in the pyranose ring and/or acetoxy side chains. The solvation shifts for protons on the ' lower side ' (α) of the ring are all less than 0.06 p.p.m. (Table 2). In comparison, the solvation shifts for (tetra-acetyl)thio-β-D-glucose alone are much less for C(2)H, C(4)H, and C(6)H although also to low field, and the largest shifts are for C(1)H and C(5)H, but to high field (Table 2). The shift of C(1)H is presumably due to H-bonding between the SH proton and the solvent. The solvation shifts for the ^{13}C resonances of the sugar in auranofin are all to low field, those for C(4) and C(6) being slightly lower than the others (Table 4).

The co-ordination shift of 0.36 p.p.m. for C(1)H on replacing H^+ of (tetra-acetyl)thioglucose by $[Au(PEt_3)]^+$ (Table 2) is similar to that for the CH proton adjacent to the thiolate S of gold thiomalate.[12] All the other proton co-ordination shifts for auranofin are small (Table 2), suggesting that gold perturbs the electron distribution of the thioglucose ligand only locally at C(1). This is confirmed by the ^{13}C shifts (see later). In most solvents the doublet for the anomeric proton, C(1)H, is clearly resolved at low field. In protic solvents the two C(6) protons and C(5)H give resolved doublets of doublets and a

Table 1. ^{1}H N.m.r. chemical shifts (δ) [a]

	Auranofin [b]						tatg [c]	
	$CDCl_3$	$(CD_3)_2SO$	$(CD_3)_2CO$	CD_3OD	CD_3OD–D_2O (1 : 1)	D_2O	$(CD_3)_2SO$	CD_3OD
C(1)H	5.119 [d]	5.207	5.192	5.177	5.252	5.349	4.963	4.756
C(2)H	5.15 [d]	4.720	4.886	4.910	4.950	5.032	4.808	4.902
C(2)Me [d]	2.016	2.008	2.009	2.042	2.111	2.142	2.022	2.051
C(3)H	5.15 [d]	5.117	5.117	5.122	5.156	5.227	5.269	5.235
C(3)Me [d]	2.058	1.977	1.987	2.034	2.105	2.109	2.017	2.036
C(4)H	4.983 [d]	4.832	4.979	5.002	5.045	5.083	4.911	5.042
C(4)Me [d]	2.083	1.964	1.975	1.989	2.060	2.075	1.981	2.002
C(5)H	3.730	3.910	3.839	3.851	3.965	4.033	4.014	3.875
$C(6)H_A$	4.224	4.085	4.192	4.259	4.338	4.396	4.125	4.249
$C(6)H_B$	4.131	3.941	4.022	4.062	4.141	4.149	4.025	4.099
C(6)Me [d]	1.988	1.904	1.913	1.940	2.017	2.027	1.940	1.966
PCH_2	1.859	1.897	1.968	1.944	1.958	1.945		
$PCCH_3$	1.226	1.135	1.236	1.246	1.238	1.212		

[a] Reference $SiMe_4$ ($Na[O_2CCH_2CH_2SiMe_3]$ in D_2O); ±0.001 p.p.m. [b] Auranofin A was used, except for D_2O measurements which refer to auranofin B. However, no differences are expected: both give the same shifts in CD_3OD. Coupling constants (Hz): $^2J[C(6)H_A$–$C(6)H_B]$ −12.2 to −12.6; $^3J[C(6)H_A$–$C(5)H]$ 3.8—5.1; $^2J(CH_2$–P) 9.8—10.3; $^3J[C(6)H_B$–$C(5)H]$ 1.8—2.4; $^3J(CH_3$–P) 18.3—19.1. [c] (Tetra-acetyl)thioglucose. [d] Ambiguous assignment.

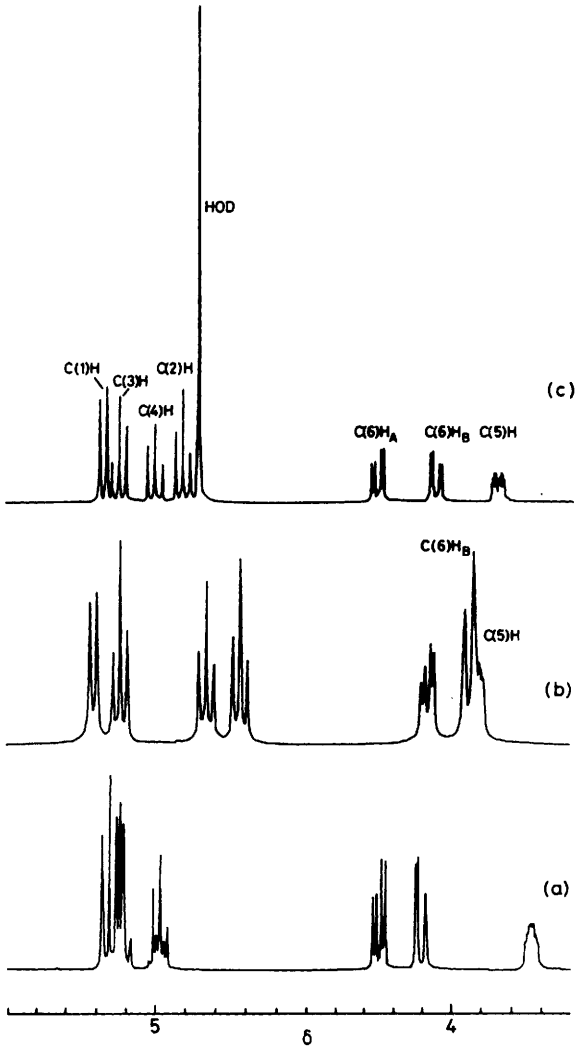


Figure 1. 400-MHz ^{1}H N.m.r. spectra of the glucopyranose ring of auranofin in (a) $CDCl_3$, (b) $(CD_3)_2SO$, (c) CD_3OD showing specific solvation shifts

Table 2. ^{1}H N.m.r. co-ordination and solvent shifts (Δδ)

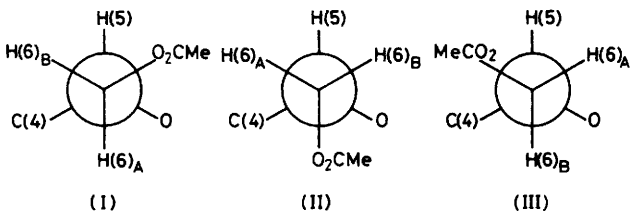
	Co-ordination shifts [a]		Solvent shifts [b]	
	$(CD_3)_2SO$	CD_3OD	Auranofin	tatg [c]
C(1)H	0.244	0.361	−0.030	−0.207
C(2)H	−0.088	0.008	0.190	0.094
C(3)H	−0.152	−0.113	0.005	−0.034
C(4)H	−0.079	−0.040	0.170	0.131
C(5)H	−0.104	−0.024	−0.059	−0.139
$C(6)H_A$	−0.040	0.010	0.174	0.124
$C(6)H_B$	−0.084	−0.037	0.121	0.074

[a] A positive value indicates a shift to high frequency (low field) on replacement of H^+ by $[Au(PEt_3)]^+$. [b] $(CD_3)_2SO$ to CD_3OD (high field shifts negative). [c] (Tetra-acetyl)thioglucose.

doublet of quartets, respectively. By selective decoupling all the peaks were assigned unambiguously except those of the acetyl methyl groups.

The three-bond ^1H–^1H couplings around the sugar ring (9.4—9.7 Hz; not tabulated) are consistent with an anti-parallel, axial–axial proton alignment. The conformation of the ring is therefore probably a chair as observed in the crystal structure.[7]

A consideration of the three-bond ^1H–^1H coupling constants provides an indication of the structural changes which occur in protic solvents leading to ^1H and ^{31}P solvation shifts. It can be seen from Table 1 that $^3J[C(6)H_A$–$C(5)H]$ decreases significantly from about 5.1 Hz in $(CD_3)_2SO$ and $(CD_3)_2CO$ to about 3.5 Hz in protic solvents such as D_2O. $^3J[C(6)H_B$—$C(5)H]$ is small (*ca.* 2.0 Hz) in most solvents. These couplings can be analysed in terms of the population of the three rotamers around the C(6)–C(5) bond [(I), (II), and (III)]. In such a system a *gauche* $^3J(^1H$–$^1H)$ coupling constant would be ex-



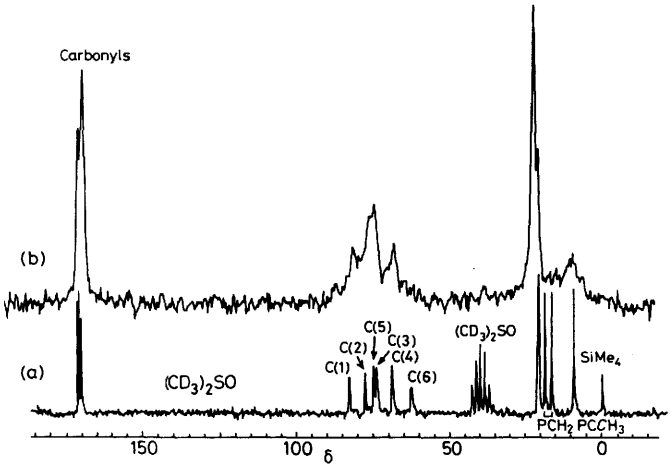


Figure 2. A comparison of the 15-MHz ^{13}C n.m.r. spectra of auranofin in (a) $(CD_3)_2SO$ and (b) solid state, with cross polarization and magic angle spinning (1 000 pulses, 5-s pulse delay, contact time 1 ms)

Table 3. ^{13}C N.m.r. chemical shifts (δ) [a]

	Auranofin			
	$(CD_3)_2SO$ [c]	CD_3OD	CD_3OD–D_2O (1 : 1)	tatg [b] CD_3OD
C(1)	81.94	84.15	83.39	75.16 (75.10)
C(2)	76.82	79.13	78.90	79.34
C(2)Me [d]	20.71	20.14	21.26	20.70
C(6)CO	169.92	172.31	174.00	172.31
C(3)	73.32	75.60	75.36	75.10 (75.16)
C(3)Me [d]	20.43	20.76	21.00	20.64
C(2)CO [d]	169.39	171.64	173.40	171.55
C(4)	68.24	70.11	69.55	69.76
C(4)Me [d]	20.27	20.64	20.82	20.59
C(3)CO [d]	169.15	171.32	172.90	171.23
C(5)	74.18	76.80	76.09	77.15
C(6)	62.23	63.71	63.33	63.36
C(6)Me [d]	20.27	20.64	20.82	20.59
C(4)CO [d]	168.69	171.32	172.90	171.23
PCH_2	17.144	18.97	18.62	
$PCCH_3$	8.85	9.55	9.58	

[a] Reference $SiMe_4$; ±0.03 p.p.m. [b] (Tetra-acetyl)thioglucose. [c] Coupling constants (Hz): ^1J(C–H) C(1) 158, C(2) 153, C(3) 151, C(4) 151, C(5) 146, C(6) 148, C(2)Me 129, C(3)Me 129, C(4)Me 130, C(6)Me 130, PCH_2 130, $PCCH_3$ 128; ^1J(P–C) P–CH_2 33; ^2J(C–CH_3) CH_3–C(2),C(3),C(4)/0.7, CH_3–C(6)O unresolved; ^3J(C–O–CH) CH–O–C(2),C(3),C(4)/0.4; CH–O–C(6)O unresolved. [d] Ambiguous assignment.

pected to be small (<5 Hz), whereas a *trans* coupling would be larger (7.5—10 Hz).[11] The couplings observed are averages determined by the populations of (I), (II), and (III). We can therefore conclude that the predominant rotamers for auranofin are (I) and (II), with little contribution from (III). In protic solvents the population of (I) decreases. In rotamer (II), the C(6) acetyl group is over the centre of the glucopyranose ring, and its movement may, in part, account for the observed solvent shifts. However, since signals for $H(6)_A$ and $H(6)_B$ cannot be unambiguously assigned, the above arguments with rotamers (I) and (III) interchanged are equally plausible.

The portion of the proton spectrum due to the phosphine ligand shows an A_3B_2X pattern similar to that observed with $[Au(PEt_3)Cl]$.[12]

Table 4. ^{13}C N.m.r. co-ordination (CD_3OD) and solvent shifts (Δδ) for auranofin

	Co-ordination shift [a]	Solvent shift [b]
C(1)	8.99	2.21
C(2)	−0.21	2.31
C(3)	0.50	2.28
C(4)	0.35	1.87
C(5)	−0.35	2.62
C(6)	0.35	1.48

[a] A positive value indicates a shift to high frequency (low field) on replacement of H^+ by $[Au(PEt_3)]^+$. [b] $(CD_3)_2SO$ to CD_3OD.

Table 5. ^{31}P Chemical shifts [a]

Solvent	δ
$CDCl_3$	38.1
C_6H_6	39.8
CD_3OD	42.7
CD_3OD–H_2O (1 : 1)	42.9
$(CD_3)_2SO$	43.7
$(CD_3)_2CO$	43.8
Phosphate buffer, pH 7 [b]	43.4
Acetate buffer, pH 4 [b]	43.3

[a] Reference 85% H_3PO_4 in D_2O. [b] Form B.

^{13}C *N.M.R. Spectra.*—The proton-decoupled ^{13}C n.m.r. spectrum of auranofin in $(CD_3)SO$ is shown in Figure 2 along with a solid-state ^{13}C n.m.r. spectrum. ^{13}C Chemical shifts and coupling constants are listed in Table 3.

The ^{13}C chemical shift assignments of auranofin were made by a combination of off-resonance and selective ^1H decoupling at 100 MHz. Selective decoupling experiments were unsuccessful at lower field strengths. In the single resonance spectrum it was clear that the lowest field carbonyl ^{13}C peak was that from the C(6) acetyl coupled not only to the CH_3 protons but also to the two protons at C(6). The remaining three carbonyl resonances were quartets of doublets. The latter, together with the four acetyl methyl peaks are the only remaining ambiguous assignments. The ^{13}C–^1H coupling constants are in the range expected for glucopyranose rings. As with $[Au(PEt_3)Cl]$, the $^1J(^{31}P–^{13}C)$ couplings but not $^2J(^{31}P–^{13}C)$ couplings are observable.

Owing to the large co-ordination shift (9 p.p.m.) for C(1), the lowest field resonance from the ring is that for C(1) in auranofin whereas it is C(2) in (tetra-acetyl)thioglucose (Table 3). The co-ordination shifts of the five remaining carbohydrate ring carbons are small, consistent with gold having little effect on the conformation or electronic structure of the ring. A similar gold-induced shift of the sulphur-bonded carbon was found for thiomalate in gold(I) thiomalate.[12]

Since auranofin is administered orally in solid form, the observation (Figure 2) that a ^{13}C spectrum can be obtained for solid samples is of interest. The spectra from the solid and the solution show a close resemblance.

^{31}P *N.M.R. Spectra.*—The undecoupled ^{31}P n.m.r. spectrum of (3) is a multiplet, due to couplings with the CH_2 and CH_3 protons of the ethyl group. The J(H–P) values derived from the proton spectra are given in Table 1. These values agree well with those of Narasimhan and Rogers [14] for triethyl-

phosphine, in which ^2J(P–H) was smaller than ^3J(P–H). Broad-band decoupling produces a single resonance. The ^{31}P chemical shift of this resonance is dependent on solvent and varies from 38.1 p.p.m. in $CDCl_3$ to 43.8 p.p.m. in $(CD_3)_2CO$, although little difference is observed between protic solvents (Table 5).

At pH 7 and 4 no change was observed in the ^{31}P n.m.r. chemical shift of auranofin for at least 10 h. Its stability at these pH values contrasts with that at pH *ca.* 1, where shifts of the resonance are observed within 4 h. A full n.m.r. study of reactions of auranofin in acidic media will be reported later.

Experimental

2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-glucopyranose was purchased from the Aldrich Chemical Co.

Preparation of Auranofin.—To a solution of (2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl) ethyl dithiocarbonate [15] (1.0 g, 2.2 mmol) in ethanol (15 cm^3) and water (10 cm^3) maintained at 0 °C was added with stirring a solution of K_2CO_3 (0.3 g, 2.2 mmol) in water (4 cm^3). After 25 min a solution of [Au(PEt_3)Cl] [13] (0.77 g, 2.2 mmol) in methylene chloride (2 cm^3) and ethanol (10 cm^3) was added. After 2 h the mixture was poured into ice–water (100 cm^3) and extracted with methylene chloride (3 × 20 cm^3); the combined extracts were washed with water (25 cm^3), dried ($MgSO_4$), filtered and evaporated *in vacuo*. Dry column chromatography [16] (silica gel, ethyl acetate) of the residue gave an oil which crystallized from methanol–water to yield auranofin (3) as needles (0.58 g, 78%), m.p. 103—105 °C; α (589.3 nm, 25 °C, 1 mol dm^{-3} in CH_3OH) 56.6 °C [lit.,[13] m.p. 110—111 °C; α (589.3 nm, 25 °C, 1 mol dm^{-3} in CH_3OH) −55.3°].

Crystal Forms.—The crystal form of auranofin (3) obtained from methanol–water is that encountered most commonly and is referred to as auranofin A. Crystals of this type, which have low solubility in water (20 μmol dm^{-3}), were used in the *X*-ray crystal structure determination.[7] A second form, auranofin B, obtained from cyclohexane–ethyl acetate (18 : 1) is sufficiently water-soluble (700 μmol dm^{-3}) to afford useful ^1H n.m.r. spectra in D_2O.

N.M.R. Measurements.—^1H N.m.r. spectra were recorded with a Varian T-60 60-MHz spectrometer, a Varian HR-220 220-MHz instrument, and a Bruker WH400 spectrometer at 400.13 MHz. ^{13}C N.m.r. spectra were measured with a Bruker WH400 instrument at 100.6 MHz, and at 50 MHz on Varian XL200 and JEOL FX200 spectrometers. The ^{13}C n.m.r. spectrum of solid auranofin A was obtained on a modified JEOL FX60 instrument by use of cross polarization and magic angle spinning. The ^{31}P resonance was recorded at 24.15 MHz using a JEOL FX60 spectrometer and at 81.03 MHz with a Bruker WM200 instrument.

The ^{31}P n.m.r. spectra were recorded in the presence of broad-band ^1H decoupling, with the solvent deuterium resonance used for the heteronuclear lock signal. The ^{31}P n.m.r. chemical shifts were measured relative to 85% H_3PO_4 in D_2O as external standard. In the ^1H and ^{13}C n.m.r. spectra the chemical shifts were measured relative to the appropriate resonance of $SiMe_4$ or $Na[O_2CCH_2CH_2SiMe_3]$. For some ^{13}C spectra the centre peak of CD_3OD or $(CD_3)_2SO$ was used as internal reference and converted to the $SiMe_4$ scale by addition of 49.0 or 39.5 p.p.m., respectively.

The solvents employed were the standard commercially available deuteriated n.m.r. solvents: $(CD_3)_2SO$, $(CD_3)_2CO$, CD_3OD, $CDCl_3$, and D_2O. The spectra were obtained at ambient probe temperature except the ^{31}P n.m.r. spectra of auranofin B, which were obtained at 37 °C.

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