Applied Organometallic Chemistry (1987) 1 355–358
0268–2605/87/01408355/$03.50

Synthesis of tri-n-butyltin taurocholate, taurodeoxycholate and glycocholate

Larry R Sherman*, Michael J Coyer* and Friedo Huber†
*Department of Chemistry, University of Scranton, Scranton, Pennsylvania 18510, USA and
†Fachbereich Chemie, Universität Dortmund, D-4600 Dortmund 50, Federal Republic of Germany

Received 25 November 1986 Accepted 17 February 1987

The bile acids, taurocholic, taurodeoxycholic and glycocholic acid, were reacted with bis(tri-n-butyltin) oxide (TBTO) to form 1:1 derivatives. The water of reaction was removed with 2,2-dimethoxypropane or by azeotropic distillation with benzene. The compounds were characterized by ^{13}C and ^{119}Sn NMR, IR, elemental analysis, molecular weight determination, DTA, TGA, and conductance measurements. The data indicated that tri-n-butyltin glycocholate forms a covalent ester, tin being tetracoordinated. The taurocholic and taurodeoxycholic acid derivatives contain one molecule of coordinated water. They partially dissociate in polar solvents. The taurocholic and glycocholic acid derivatives were tested *in vitro* as anticancer agents and exhibited ED$_{50}$ in the 0.2–0.4 ppm (mg kg^{-1}) range against KB epidermoid tumor and P-388 leukemia using NCI protocols.

Keywords: Tri-n-butyltin taurocholate, tri-n-butyltin taurodeoxycholate, tri-n-butyltin glycocholate, bis(tri-n-butyltin) oxide, synthesis

INTRODUCTION

Tri-n-butyltin fluoride (TBTF) had been reported to be an anticancer agent.[1] However, it was felt that the compound by itself should not exhibit these properties, and it was postulated that TBTF might react with the bile acids in the duodenum of the intestine to form esters which could be carried to the tumor by the bloodstream.[2] However, organotin derivatives of bile acids are not known, and other tin steroid derivatives have only recently been reported in the literature.[3–5] These compounds of cholesterol, cholic acid or hormones, on the whole, are either ether-like or ester-like in their bonding,[3,5] but adducts also have been identified.[4] Extending our previous work we have now synthesized tri-n-butyltin derivatives of glycocholic acid (GA; Chem. Registry No. 863-57-0), taurocholic acid (TA; Chem. Registry No. 81-24-3), and taurodeoxycholic acid (TDA; Chem. Registry No. 516-50-7)—steroids containing an active side chain. The synthesis was accomplished with established procedures for preparing alkyltin carboxylates[6] and sulfonates.[7,8]

EXPERIMENTAL

Chemicals and instruments

Bis(tri-n-butyltin) oxide (TBTO; Chem. Registry No. 56-35-9) was obtained from Schering AG, Bergkamen, FRG; GA and the sodium salts of TA (Chem. Registry No. 145-42-6) and TDA (Chem. Registry No. 1180-95-6) were from Sigma Chemicals, St Louis, MO, USA. The elemental analyses were performed on a Carlo Erba Strumentazione Elemental Analyzer 1106, the DTA and TGA on a Mettler Vakuum Thermoanalyzer TA 1, and the molecular weight measurements on a Knauer Dampfdruck Osmometer. The IR spectra (KBr pellet or nujol mull) were recorded with a Perkin–Elmer 580B spectrophotometer using CsBr windows, the NMR spectra on a Bruker AM 300, and conductivity values were measured with a WTW LF 530 conductivity meter.

Syntheses

Stoichiometric quantities of TBTO (0.831 g) and GA (1.30 g) were dissolved in 20 cm^3 methanol and refluxed for 24 h with 2 cm^3 of 2,2-dimethoxypropane (DMP). After distilling off the solvent with a rotary evaporator, the unreacted TBTO was removed with two 10 cm^3 pentane extractions and the unreacted GA with a 5 cm^3

water extraction. The resulting white powder, tri-n-butyltin glycholate (TBT-GA) had a m.p. of 112–113°C using a standard capillary-tube m.p. apparatus. The yield was 90%. The product was soluble in alcohols, ketones and chlorinated hydrocarbons, but not in water or hydrocarbons.

Sodium salts of TA and TDA (1.5 g) were dissolved in 10 cm^3 of methanol and converted to the free acids with an H$^+$ cation exchange resin (Amberlite IR-120). The resulting methanol solutions of the acids were refluxed for five days with 0.815 g of TBTO in the presence of 6 cm^3 of DMP. After removing the solvents with a rotary evaporator, the unreacted TBTO was removed with several pentane extractions. The impure product was dissolved in 5 cm^3 of methanol and the unreacted acids and impurities were absorbed on an 8 cm Al_2O_3 column using 30 cm^3 of methanol as the eluant. The impurities remained on the column and a white crystalline solid was obtained after removing the solvent. The resulting white crystalline products, tri-n-butyltin taurocholate (TBT-TA) and tri-n-butyltin taurodeoxycholate (TBT-TDA) did not have a true melting point; they appeared to dehydrate near 140°C and decomposed at 220°C. The yield in both cases was 60%. The products were soluble in water and polar solvents but not in saturated hydrocarbons or chlorinated hydrocarbons.

TBT-TA was also synthesized from 1.28 g of TBTO and 1.12 g of free taurocholic acid by azeotropic distillation with benzene. The resulting product was soluble in benzene and was cleaned up as described above. The yield was 58% and the product had the same characteristics as previously described.

Conductance measurements

The conductance of all three compounds was measured at approximately 1.50 and 0.75 mmol dm^{-3} solutions in DMF at 20°C. A solution of TBT-GA gave a value (average 38 ohm^{-1} cm^2) for both concentrations or slightly less than that for pure DMF (46 ohm^{-1} cm^2) indicating that the molecule is not dissociated. Solutions of TBT-TA and TBT-TDA gave values of approximately 40 and 18 ohm^{-1} cm^2 for the two concentrations, respectively, after correcting for the solvent. Normalizing the data to 1.0 mmol dm^{-3} yielded a value of 29 ohm^{-1} cm^2/mmol dm^{-3} for both compounds. The value is about half of the conductance recommended by Geary[9] for a 1:1 ionization of an inorganic complex in DMF. If the criteria put forward by Geary can be applied to these compounds, the data would indicate incomplete ionization of TBT-TA and TBT-TDA. When corrected for the DMF blank, a plot of conductance versus concentration yielded a straight line for the three compounds. The concentration of TBT-GA in the plot was assumed to be zero, i.e. ionization of the form shown below for TBT-TA can be excluded (Eqn 1).

$$\text{TBT-TA} \rightleftharpoons \text{TBT}^+ + \text{TA}^-. \qquad [1]$$

RESULTS

Elemental analyses, DTA, TGA and molecular weight

The DTA and TGA for TBT-GA confirmed a melting point of 113°C and indicated decomposition above 240°C. The DTA and TGA for TBT-TA and TBT-TDA confirmed a dehydration at 140°C and decomposition at 220°C. They also indicated one molecule of water per TBT-TDA and per TBT-TA molecule. The elemental analyses were calculated using these data and are presented in Table 1. The data for C, H, N and Sn are within acceptable limits of deviation. The tin was gravimetrically determined as SnO_2 after oxidizing the organic portion of the molecule with H_2SO_4/HNO_3 and heating to 700°C. The vapor-phase osmometry data for TBT-GA in $CHCl_3$ yielded 758 versus 754, indicating a monomeric molecule. For TBT-TDA in DMF, they yielded 404 versus 805 and indicated that the TBT-TDA dissociates in DMF. For TBT-TA in DMF, a value of 392 versus 823 was obtained. This result indicated that TBT-TA also dissociates.

Infrared and nuclear magnetic resonance data

The IR spectrum of TBT-GA showed a loss of the acid carbonyl band at 1735 cm^{-1}, a new C=O band at 1540 cm^{-1} and a shift in the C—O band from 1235 to 1270 cm^{-1} indicating an ester linkage. The rest of the bands were fingerprint bands of GA, except those at 1470, 680 and 700 cm^{-1} which were practically the same as in the IR spectra of TBTO and tri-n-butyltin chloride (TBTCl). The NMR spectrum yielded a strong ^{119}Sn singlet at 69.93 ppm with coupling

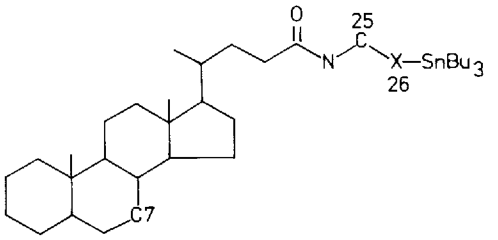
Table 1 Analytical data for tri-n-butyltin derivatives of bile acids

	Melting point (°C)	Analysis: Found (Calcd) (%)				Mol. wt: Found (Calcd)
		C	H	N	Sn	
TBT-GA $C_{38}H_{69}NO_6Sn$	113	60.3 (60.4)	9.3 (9.7)	1.9 (1.9)	16.2 (15.7)	758 (755)
TBT-TA.H_2O $C_{38}H_{73}NO_8SSn$	140 (d220)[a]	55.4 (55.5)	9.1 (8.9)	2.1 (1.70)	14.60 (14.40)	392 (823)
TBT-TDA.H_2O $C_{38}H_{73}NO_7SSn$	140 (d220)[a]	56.6 (56.6)	8.2 (9.2)	1.8 (1.7)	15.12 (15.05)	404 (807)

[a] d, Decomposition.

constants $^1J(^{119}Sn^{13}C)$ of 353.5, 20.2 and 63.6 Hz for the C_1, C_2 and C_3 butyl carbons, respectively (see Fig. 1). A complete assignment of the ^{13}C peaks is given in Table 2.

The IR spectra of TBT-TA and TBT-TDA showed fewer characteristics. The OH band at 3160–3560 cm^{-1} was broader, indicating hydrogen bonding. The remainder of the bands were



	C(7)	C(25)	X(26)
TBT-GA	$>$CHOH	$-CH_2-$	$-\overset{\overset{\displaystyle O}{\|}}{C}(26)-O-$
TBT-TA	$>$CHOH	$-CH_2-$	$-C(26)H_2-\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle O}{\|}}{S}}-O-$
TBT-TDA	$>CH_2$	$-CH_2-$	$-C(26)H_2-\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle O}{\|}}{S}}-O-$

Table 2 ^{13}C NMR assignments for bile acid/TBT compounds in ppm

Carbon	TBT-GA	TBT-TA	TBT-TDA
Steroid			
1	35.38	35.79	35.86
2	31.51	30.74	30.44
3	71.81	71.04	71.20
4	41.95	39.98	36.50
5	41.55	41.90	41.83
6	34.74	34.96	27.20
7	68.37	66.87	27.39
8	39.49	40.26	40.26
9	27.55	26.76	34.02
10	34.74	34.96	35.26
11	30.33	28.96	28.80
12	73.08	71.68	70.15
13	46.63	46.32	47.64
14	41.55	42.03	46.19
15	23.24	23.43	23.72
16	28.08	27.88	26.31
17	46.37	46.70	48.80
18	12.43	12.90	12.64
19	22.43	23.14	23.30
20	35.44	31.27	35.60
21	17.39	17.66	17.27
22	34.62	33.21	32.80
23	33.09	32.18	31.74
24	174.41	173.53	172.31
25	46.37	46.32	46.19
26	173.74	51.04	50.82
n-Butyl groups at Sn			
1′	16.71	18.80	19.42
2′	27.68	28.17	27.76
3′	26.95	27.22	26.73
4′	13.50	14.17	13.78

the same as for the starting materials except the bands at 910 and 980 $\mathrm{cm^{-1}}$ and at 1470, 1070, 680 and 700 $\mathrm{cm^{-1}}$, which are similar to those in TBTO and TBTCl as mentioned earlier. No distinct differences could be distinguished between the KBr and mull spectrograms. The lack of change in IR bands of the sulfonic acid group was an indication that the nature of the bonding was similar in starting materials and products. The NMR spectrum for the TA and TDA derivatives yielded a strong ^{119}Sn singlet at 701.2 and 699.1 ppm, respectively; however, the coupling constants were not resolved. A complete assignment of the ^{13}C peaks is given in Table 2.

DISCUSSION

TBT-GA appears to be a covalent compound in the solid state and in solution with the Bu_3Sn group attached through an ester like bond to the carboxylate group X(26) (Fig. 1). From the $\Delta\nu$ value[10,11] of 270 $\mathrm{cm^{-1}}$ for the $\nu_{asym}(CO_2)$ and the $\nu_{sym}(CO_2)$, a unidentate coordination of the carboxylate group to tin is inferred, and, consequently, a tetracoordinated tin species.

In contrast to TBT-GA, the two sulfonates TBT-TA and TBT-TDA dissociate partially in polar solvents, as indicated by molecular weight and conductance data. The similarity of TBT-TA and TBT-TDA is readily understandable when one realizes that these two compounds differ only in the presence of an OH group on C(7) of TA. This OH group has little or no influence on the bonding of the TBT moiety. The water molecules are probably attached to the Sn atom as observed in other tin sulfonic acid derivatives.[8]

In vitro testing

Both TBT-TA and TBT-GA were tested *in vitro* as antitumor agents against the human KB epidermoid tumor and mouse P-388 leukemia by Dr W. Lichter, University of Miami, School of Medicine, Miami, FL, using NCI protocol. The evaluation unit is the ED_{50} (Inhibition Index). The ED_{50} is the concentration of the chemical at which 50% of the cells in the culture are no longer capable of mitotic division. For TBT-TA the ED_{50} was 0.2 and 0.3 ppm ($\mathrm{mg\,kg^{-1}}$) respectively, and for TBT-GA it was 0.2 and 0.4 ppm ($\mathrm{mg\,kg^{-1}}$). Values of 1 ppm or less warrant further investigation.

Acknowledgements The authors wish to acknowledge financial support from the Gustus and Louise Pfeiffer Foundation, the University of Scranton Research Fund and a gift of TBTO from Schering AG, Bergkamen. They also wish to thank Dr Robert Rüther (University of Dortmund) for his helpful advice and criticism.

REFERENCES

1. Cardarelli, N F, Quitter, B M, Allen, A, Dobbins, E, Libby, E P, Hager, P and Sherman, L R *Aust. J. Exp. Biol. Med. Sci.*, 1984, 62: 199
2. Sherman, L R, Second Int. Symp. Tin Upon Malignant Cell Growth, Chapman Lake, PA, May 20–22, 1985
3. Cardarelli, N F and Kanakkanat, S V *US Patent* 4 541 956, Sept. 17, 1985
4. Saxena, A, Huber, F, Pellerito, L, Girasolo, A and Stocco, G C *Inorg. Chim. Acta*, in press
5. Saxena, A, Huber, F, Pellerito, L and Girasolo, A *Appl. Organomet. Chem.*, submitted for publication
6. Bähr, G and Pawlenko, S Methoden zur Herstellung und Umwandlung von Organo-zinn-Verbindungen. In: Houben-Weyl *Methoden der Organischen Chemie*, 4th Edn, Vol. 13/6, Thieme, Stuttgart, 1978, pp 181–521
7. Anderson, H H *Inorg. Chem.*, 1964, 3: 108
8. Harrison, P G, Phillips, R C and Richards, J A *J. Organomet. Chem.*, 1976, 114: 47
9. Geary, W J *Coord. Chem. Rev.*, 1971, 7: 81
10. Deacon, G B and Phillips, R J *Coord. Chem. Rev.*, 1980, 33: 227
11. Deacon, G B, Huber F and Phillips, R J *Inorg. Chim. Acta*, 1985, 104: 41