Semi-synthetic Antibiotics: Titanium(IV), Zirconium(IV) and Mercury(II) Complexes of 6-Amino Penicillinic Acid*†

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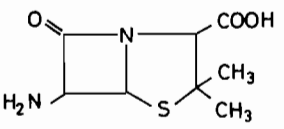
Abstract

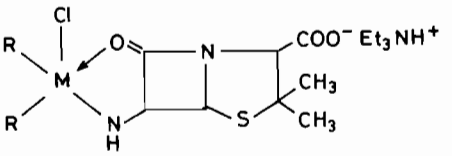
A number of organometallic derivatives involving 6-amino penicillinic acid (I), of the types $(\eta^5\text{-R})_2\text{M(Cl)L}^-\text{Et}_3\text{NH}^+$ (II), $(\eta^5\text{-R})_2\text{M(Cl)L}$ (III) and R'HgL [R = cyclopentadienyl (C_5H_5), indenyl (C_9H_7), R' = phenyl (C_6H_5), *p*-acetoxyphenyl (*p*-$CH_3COOC_6H_4$), *o*-hydroxyphenyl (*o*-HOC_6H_4), *p*-hydroxyphenyl (*p*-HOC_6H_4); M = Ti(IV), Zr(IV); LH = 6-amino penicillinic acid] have been synthesized and characterized. Conductance measurements indicate that while the $(\eta^5\text{-R})_2\text{M(Cl)L}^-\text{Et}_3\text{NH}^+$ complexes are 1:1 electrolytes, the remaining compounds are non-electrolytes. From IR and UV spectral studies it is concluded that the penicillin moiety is bidentate. PMR and CMR studies support the stoichiometry of the complexes. Fluorescence studies have been carried out for *o*- and *p*-HOC_6H_4HgL complexes and relevant photochemical parameters have been elucidated. X-ray diffraction studies have been made for the *o*-HOC_6H_4HgL complex. For the C_6H_5HgL, *p*-$CH_3COOC_6H_4HgL$ and *p*-HOC_6H_4HgL complexes, thermal studies (TG and DTA) have been carried out and kinetic parameters for thermal degradation have been enumerated. In addition, the fragmentation pattern of these complexes has been analysed on the basis of mass spectra. The C_6H_5HgL and *p*-$CH_3COOC_6H_4HgL$ complexes show positive bactericidal activities.

Introduction

In an earlier communication [1] we gave a brief report of our investigations on organometallic derivatives of 6-amino penicillinic acid. Our interest in the study of such complexes is mainly due to the fact that the introduction of substituents into the penicillin nucleus effects changes in antibiotic activity and β-lactamase susceptibility. Since the introduction of penicillins into clinical practice a continual problem has been the emergence of drug resistant strains of bacteria. In many cases this resistance arises from the production of β-lactamases, enzymes which degrade these antibiotics. The synthesis of new penicillins is important to keep pace with the appearance of these resistant bacterial strains. With this aim we carried out the synthesis and characterisation of a number of organo titanium(IV), zirconium(IV) and mercury(II) complexes of 6-amino penicillinic acid. This is the first detailed report on organometallic substituted semi-synthetic antibiotics in which the penicillin moiety is directly bound to the metal ion.



I



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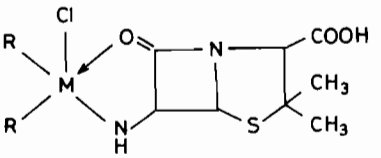

III

R = C_5H_5, C_9H_7
M = Ti(IV), Zr(IV)

*Some of the results have been presented in a preliminary form (ref. 1).

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Experimental

The following instruments were used: Elico conductivity Bridge, Model CM-82 for conductance measurements; Perkin-Elmer grating 621 spectrometer for IR spectra; Perkin-Elmer UV-vis spectrophotometer, model 554 for UV spectra; Perkin-Elmer R-32 spectrometer for PMR spectra; Jeol FX-200 spectrometer, Japan for CMR spectra; Jasco FP-550 spectrofluorometer for fluorescence studies; Philips X-ray diffractometer for X-ray diffraction; G-70 thermoanalyser, SETARAM, Lyon, France for TG studies in air at a heating rate of 8 °C min^{-1}; Mettler TA-20 for DTA studies in air at a heating rate of 8 °C min^{-1} and chart speed 30 cm h^{-1}. Mass spectra were recorded at the central Drug Research Institute, Lucknow (India).

Nitrobenzene was purified for conductance measurements by the method of Fay *et al.* [2]. $(\eta^5\text{-}C_9H_7)_2TiCl_2$ [3], $(\eta^5\text{-}C_9H_7)_2ZrCl_2$ [3], C_6H_5HgCl [4], *o*-, *p*-HOC_6H_4HgCl [5] and *p*-$CH_3COOC_6H_4HgCl$ [6] were prepared by standard methods. $(\eta^5\text{-}C_5H_5)_2TiCl_2$ and $(\eta^5\text{-}C_5H_5)_2ZrCl_2$ were purchased from Fluka AG, Switzerland and Alfa Inorganics Ventron, USA, respectively.

Preparation of $(\eta^5\text{-}R)_2M(Cl)L^-Et_3NH^+$ Complexes

A solution of $(\eta^5\text{-}R)_2MCl_2$ (0.50 mmol) in 25 ml THF was added slowly to a suspension of 6-aminopenicillinic acid (0.50 mmol) in 25 ml THF and 10 ml triethylamine. The contents were stirred for about 2 h at room temperature and filtered. The filtrate was evaporated to dryness under vacuum. The solid product was washed successively with dichloromethane and petroleum ether and dried *in vacuo*.

Preparation of $(\eta^5\text{-}R)_2M(Cl)L$ Complexes

The $(\eta^5\text{-}R)_2M(Cl)L^-Et_3NH^+$ complexes, obtained by the above method were dissolved in the minimum quantity of water and 5 ml of 2 M HCl was added. The $(\eta^5\text{-}R)_2M(Cl)L$ complexes precipitated. These were washed with petroleum ether and recrystallised from acetone.

Preparation of R'HgL Complexes

A solution of R'HgCl (0.50 mmol) in 25 ml THF was added slowly to a suspension of 6-aminopenicillinic acid (0.50 mmol) in 25 ml THF and 10 ml triethylamine. The contents were stirred for about 2 h at room temperature and then filtered. The filtrate was evaporated to dryness under vacuum. The solid product was suspended in about 10 ml water and 5 ml of 2 M HCl was added. The R'HgL complexes precipitated. These were washed with petroleum ether and recrystallised from acetone.

Results and Discussion

The complexes are yellow or brown in colour. They are soluble in THF, DMSO and acetone. The $(\eta^5\text{-}R)_2M(Cl)L^-Et_3NH^+$ complexes are soluble in water too. Conductance measurements for $(\eta^5\text{-}R)_2M(Cl)L^-Et_3NH^+$ complexes reveal that these are 1:1 electrolytes in nitrobenzene. The remaining compounds are non-electrolytes. With ninhydrin reagent, the complexes give violet coloration characteristic of amino acids. From TLC and elemental analyses it is concluded that the compounds are pure. Some physical characteristics and elemental analysis data are presented in Table I.

TABLE I. Physical Characteristics and Elemental Analyses.

Compound	Dec. Temp. (°C)[a]	Λ[b] ($C = 1.5 \times 10^{-3}$ M)	Found (calc.) %			
			M	Cl	S	N
$(C_5H_5)_2Ti(Cl)L^-Et_3NH^+$	147	28.8	9.19(9.04)	6.75(6.69)	6.15(6.05)	7.78(7.93)
$(C_5H_5)_2Zr(Cl)L^-Et_3NH^+$	140	29.2	16.10(15.92)	6.00(6.19)	5.69(5.58)	7.48(7.33)
$(C_9H_7)_2Ti(Cl)L^-Et_3NH^+$	98	26.6	7.50(7.61)	5.75(5.63)	5.18(5.08)	6.53(6.67)
$(C_9H_7)_2Zr(Cl)L^-Et_3NH^+$	155	30.2	13.69(13.56)	5.38(5.27)	4.82(4.75)	6.40(6.24)
$(C_5H_5)_2Ti(Cl)L$	123	0.52	11.27(11.18)	8.39(8.28)	7.32(7.47)	6.70(6.53)
$(C_5H_5)_2Zr(Cl)L$	118	0.52	19.48(19.34)	7.62(7.51)	6.65(6.78)	5.80(5.94)
$(C_9H_7)_2Ti(Cl)L$	120	0.58	9.18(9.06)	6.83(6.71)	6.13(6.05)	5.45(5.30)
$(C_9H_7)_2Zr(Cl)L$	113	0.46	15.82(15.95)	6.35(6.20)	5.65(5.59)	4.75(4.89)
C_6H_5HgL	95[c]	0.54	40.88(40.72)	–	6.39(6.49)	5.50(5.68)
p-$CH_3COOC_6H_4HgL$	152	0.48	36.53(36.43)	–	5.72(5.81)	5.21(5.08)
o-HOC_6H_4HgL	78	0.46	39.56(39.44)	–	6.17(6.29)	5.65(5.50)
p-HOC_6H_4HgL	93[c]	0.50	39.33(39.44)	–	6.38(6.29)	5.72(5.50)

[a] Uncorrected values. [b] In Ohm^{-1} cm^2 mol^{-1}. [c] Melting point.

IR Spectra

The IR spectra of penicillin and its derivatives and possible models of penicillin have been extensive [7]. In the case of 6-amino penicillinic acid, the carbonyl absorption frequency of the lactam ring is centered at 1765 cm^{-1}. However, for the metal complexes, it is shifted to 1650–1700 cm^{-1}, indicating that the carbonyl group is bound to the metal ion. Thus the penicillin moiety is bidentate. The C–N stretching frequency in the complexes absorbs at ~1040 cm^{-1}. This absorption appears as a doublet. In the case of 6-amino penicillinic acid, the corresponding absorption occurs at 1015 cm^{-1}. The higher frequency observed in the case of metal complexes is due to the transfer of charge from the ligand to the metal. The C–S stretching frequency absorbs at ~580 cm^{-1}.

In the case of $(\eta^5\text{-R})_2M(Cl)L^-Et_3NH^+$ complexes, strong bands at ~1600 cm^{-1} and ~1300 cm^{-1} indicate the presence of the ionic carboxylic group. This is supported by the fact that the OH deformation vibration of the carboxylic group, which is expected to occur at ~935 cm^{-1} [8], is absent in these cases. In $(\eta^5\text{-R})_2M(Cl)L$ and R'HgL complexes, the bands which appeared at ~1600 cm^{-1} and ~1300 cm^{-1} in the previous case were replaced by a strong band at ~1250 cm^{-1} and a weak band at ~1400 cm^{-1} indicative of the non-ionic carboxylic group [9]. Further, the OH deformation vibration is observed at ~935 cm^{-1} in these cases.

The band at ~2715 cm^{-1}, observed in the case of $(\eta^5\text{-R})_2M(Cl)L^-Et_3NH^+$ complexes is indicative of the presence of the Et_3NH^+ group [9]. The C–H aromatic stretching frequency of the π-ring systems absorbs at ~2930 cm^{-1}. Two prominent bands are also observed at ~2660 cm^{-1} and ~2480 cm^{-1}.

UV Spectra

The UV spectrum of 6-amino penicillinic acid shows a very intense band at 210 nm (log ϵ 4.8) due to the $\pi-\pi^*$ absorptions of the chromophoric C⋯O group. In the case of the Ti(IV) and Zr(IV) complexes, this band is shifted to *ca.* 224 nm while in the case of the Hg(II) complexes it appears at *ca.* 242 nm, although its intensity in each case remains virtually the same. The shift is attributed to the involvement of the lactam C⋯O group in complexation, thus supporting the conclusions drawn from IR studies.

PMR and CMR Spectra

The PMR spectra showed the following signals for the penicillin moiety: δ 5.30 (2H, s, methine at C_5 and C_6); δ 4.20 (1H, s, methine at C_3); δ 1.62 (3H, s, methyl); δ 1.48 (3H, s, methyl). The cyclopentadienyl protons absorb as a singlet at δ 6.0 and the indenyl protons appear as a multiplet at δ 6.8–7.3 similar to the values observed for other chelates involving these groups [10].

The CMR spectrum of 6-amino penicillinic acid was recorded in D_2O + HCl, and that of the complexes in d_6-DMSO. The spectrum of 6-amino penicillinic acid shows signals at 168.295(s) and 167.991-(s) ppm due to the carboxylic group or lactam carbonyl. As such it is difficult to identify both of these groups. However, in the metal complexes the low field signal is shifted further downfield by about 2 ppm indicating the involvement of this group in complexation. Hence this signal is due to the lactam carbonyl group.

Similarly the C_3 and C_6 carbons in 6-amino penicillinic acid absorb at 71.4289(d) and 73.612(d) ppm. Since the latter value in the complexes is shifted further downfield by about 1 ppm, it is identified with C_6 carbon. The data for the remaining carbons are: C_7, 61.187(d); C_2, 53.146(d); CH_3, 26.869(q), 28.186(q) ppm.

Fluorescence Studies

The compounds $o\text{-}HOC_6H_4HgL$ and $p\text{-}HOC_6H_4$-HgL are fluorescent in nature. Hence fluorescence studies have been carried out for them. In accordance with the Franck-Candon principle and thermal relaxation of vibrational modes, the fluorescence spectrum is observed on the red side of the absorption spectrum in approximately mirror-image relationship [11]. The spectrum is free from anti-Stokes effects. The pattern of the spectrum follows Levschin's rule, indicating that the geometry of the excited state is not very different from that of the ground state [12].

The actual radiative lifetime of the excited state, τ is smaller than the intrinsic radiative life time, τ_0, indicating the possibility of non-radiating energy dissipation processes depopulating the excited state. Thus, fluorescence remains the dominant but certainly not the exclusive mode of emission. The non-radiative processes, *i.e.* inter-system crossing and internal conversion, compete with fluorescence. Therefore, Einstein's probability of spontaneous absorption, Bnm, exceeds the corresponding probability of spontaneous emission, Amn. The quantum yield of fluorescence, inter-system crossing and internal conversion follow the order $\phi_f > \phi_{ISC} > \phi_{IC}$. These priorities are also established by their respective rate constants [13].

The summation of the rate constants for all the photochemical and photophysical processes competing with fluorescence, ΣK_i, equals the sum of K_{ISC} and K_{IC}. Thus apart from fluorescence, inter-system crossing and internal conversion, there seems to be no other mode of emission, radiative or non-radiative, in the present case [12]. The oscillator strength, f, has been calculated from the relation $f = 4.31 \times 10^{-9} \int \epsilon \bar{\nu} d\bar{\nu}$, which is valid if we assume a Lorenzian shape for the absorption band. The factor

TABLE II. Fluorescence Spectral Parameters.

Parameter	o-HOC_6H_4HgL	p-HOC_6H_4HgL
Quantum yield of fluorescence: ϕ_f	0.66	0.62
Quantum yield for inter-system crossing: ϕ_{ISC}	0.33	0.37
Quantum yield for internal conversion: ϕ_{IC}	0.007	0.009
Oscillator strength: f	0.012	0.013
Actual radiative lifetime: τ (s)	4.5×10^{-8}	5.8×10^{-8}
Intrinsic radiative lifetime: τ_0 (s)	6.8×10^{-8}	9.3×10^{-8}
Rate const. of fluorescence emission: K_f (s^{-1})	0.14×10^8	0.10×10^8
Rate const. for inter-system crossing, $S_1 \rightarrow T_1$: K_{ISC} (s^{-1})	0.072×10^8	0.061×10^8
Rate const. for internal conversion: K_{IC} (s^{-1})	0.0015×10^8	0.0016×10^8
Einstein's absorption probability: B_{nm}	0.39×10^8	0.41×10^8
Einstein's emission probability: A_{mn}	0.15×10^8	0.15×10^8

$\int \epsilon \bar{\nu} d\bar{\nu}$ is replaced by $\epsilon_{max}\ \Delta\bar{\nu}$, where $\Delta\bar{\nu}$ is the half band width of the absorption band [12]. The relevant data are presented in Table II.

X-ray Diffraction

The X-ray powder diffraction spectrum for the o-HOC_6H_4HgL complex has been studied using CuKα radiation with λ = 1.54178 Å. The complex gives a poor diffraction photograph, with diffuse background scatterings. The following d spacings have been identified: 9.82 m, br; 8.75 s; 7.89 m; 7.83 br; 7.43 s; 6.51 w; 5.09 m; 4.62 w; 3.41 m; 2.61 m, br; 2.39 m, br.

Thermal Studies

Thermogravimetric (TG) studies have been carried out for C_6H_5HgL, p-$CH_3COOC_6H_4HgL$ and p-HOC_6H_4HgL complexes. The weight loss from the TG curve, in each case, corresponds to the formation of HgO, which subsequently volatilizes, leaving the crucible of the thermobalance empty. The order (n) and activation energy (E_a) of the thermal decomposition reaction have been elucidated by the method of Coats and Redfern [14].

The order of reaction in each case is one. The activation energies in Kcal mol^{-1} for the thermal decomposition of p-$CH_3COOC_6H_4HgL$, C_6H_5HgL and p-HOC_6H_4HgL are 10.06, 18.30 and 24.40 respectively. The p-$CH_3COOC_6H_4HgL$ complex has the lowest value for E_a. This may be explained by the electron withdrawing effect of the acetoxy group, which leads to a weakening of the Ar–Hg bond, thus making thermal degradation relatively easy. In the case of the p-HOC_6H_4HgL complex, the phenolic group is electron donating and the Ar–Hg bond is strengthened. Therefore, the value of the activation energy in this case is higher than in the unsubstituted C_6H_5HgL complex. That Ar–Hg bond cleavage is involved in the pyrolysis of the complexes is also indicated by the mass spectra, where peaks for $C_6H_5^+$, $CH_3COOC_6H_4^+$ and $HOC_6H_4^+$ have been observed.

The mechanism of the thermal reaction has been elucidated by the method of Satava [15]. In this method, the function $f(\alpha)$, which depends upon the mechanism, is given by $\int f(\alpha)^{-1}\, d\alpha = g(\alpha)$, where α is the fraction decomposed at temperature T_α. For the correct mechanism, log g (α) must be a linear function of $1/T_\alpha$. In the present cases it has been observed that only the curve corresponding to the F_1 mechanism is a straight-line. For the F_1 mechanism, the rate equation is: $-\ln(1 - \alpha) = Kt$ (where K is the rate constant and t is time) and the rate controlling process is random nucleation.

The TG data are supplemented by differential thermal analysis (DTA) studies. The thermal effects on DTA curves are exclusively endothermic in nature. The activation energy (E_a) for the first thermal effect in each case has been determined [16]. For the calculation of heat of transition (ΔH) [17], the temperature dependent calibration coefficient was obtained from the Currell equation [18]. The T_{max} (K), E_a (Kcal mol^{-1}) and ΔH (cal g^{-1}) data are shown in Table III.

TABLE III. T_{max} (K), E_a (Kcal mol^{-1}) and ΔH (cal g^{-1}) Data.

Complex	T_{max}	E_a	ΔH
p-$CH_3COOC_6H_4HgL$	631	41.18	56.56
C_6H_5HgL	365	54.91	51.50
p-HOC_6H_4HgL	363	60.99	53.78

Mass Spectra

The mass spectra recorded for the C_6H_5HgL, p-$CH_3COOC_6H_4HgL$ and p-HOC_6H_4HgL complexes indicate that the Ar–Hg bond is cleaved resulting in Ar^+ and HgL^+ fragments. Thus the peaks with m/e 77, 135 and 93 correspond to the formation of $C_6H_5^+$, $CH_3COOC_6H_4^+$ and $HOC_6H_4^+$ fragments respectively. The HgL^+ fragment is transformed to

$(L\text{-}CH_3)^+$ with m/e 200. The latter fragment undergoes loss of the second methyl group and then the carboxylic groups and the corresponding peaks are observed at m/e 185 and 140.

Biochemical Studies

The complexes C_6H_5HgL and $p\text{-}CH_3COOC_6H_4HgL$ were tested for antibacterial activity *in vitro*. The screening of the samples was carried out against three bacteria, *viz.* gram-positive *Staphylococcus aureus* (*S. aureus*), gram negative *Pseudomonas aeruginosa* (*Ps. aeruginosa*) and gram negative *Escherichia coli* (*E. coli*) and at two concentrations, *viz.* 50 μg/ml and 100 μg/ml. The compounds were inactive at lower concentrations against *E. coli*, but were active against it at higher concentrations. For the remaining bacterial strains, both compounds revealed significant inhibition. The samples were equally active against *Ps. aeruginosa* at both concentrations. Against *S. aureus*, the compounds were more active at 100 μg/ml than at 50 μg/ml. The order of activity against the three microorganisms is *Ps. aeruginosa* > *S. aureus* > *E. coli*.

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