H, OH, COOH), 7.40–6.66 (m, 4 H, ArH), 3.23 (d, 1 H, CHCOO), 2.27–0.5 (m, 9 H, $CHCH_3CH_2CH_3$).

3-Substituted Phenylacetyl Chloride (XVI).—To a soln of 3-substituted phenylacetic acid (1 mole) in dry Et_2O was added $SOCl_2$ (3 moles), and the mixture was refluxed for 3 hr. Evaporation of the solvent gave the crude chloride (XVI) which was used for the following reaction without purification.

Haloalkyl 3-Substituted Phenylacetate (XVII).—To a soln of chloride XVI (1 mole) in dry PhH was added a mixture of haloalkylcarbinol (1.1 moles) and pyridine (1 mole) and the resultant mixture was refluxed for 5 hr. After the evapn of the solvent, the resulting residue was extd (Et_2O) after addn of H_2O. The extract was washed (H_2O), dried (Na_2SO_4), and evapd. The residual oil was distd *in vacuo* to give XVII.

Amino 3-Substituted Phenylacetates (I). General Procedure. A.—A mixture of carboxylic acid (1 mole), aminoalkyl chloride (1.1 moles), and NaOEt (prepared from 1.1 g-atoms of Na) in EtOH was refluxed for 3 hr, and the solvent was evapd. The resulting residue was extd (Et_2O) after addition of H_2O. The extract was washed (H_2O), dried (Na_2SO_4), and evapd to give the crude compd I, which was purified by distillation *in vacuo*, or column chromatography, or formation of salt such as the oxalate.

B.—A mixture of acid halide (XVI, 1 mole), pyridine (1 mole), and aminoalkylcarbinol (1.1 moles) in PhH was refluxed for 5 hr. The solvent was evapd and the resulting residue was extd (Et_2O) after the addn of H_2O and 5% NaOH. Work-up as in method A gave I.

C.—A mixture of the ester (XVII, 1 mole) and secondary amine (2 moles) was heated on a water bath for 5 hr. Treatment as in B gave I.

O-Methylation of 3-Hydroxy Derivatives.—To a soln of aminoalkyl 3-hydroxyphenylacetate in Et_2O was added excess CH_2N_2 in Et_2O and the mixture was allowed to stand in the refrigerator for 3 days. Evaporation of the solvent, followed by purification by column chromatography on silica gel, gave the 3-methoxy derivatives.

Diethylaminoethyl 2-(3-Acetoxyphenyl)phenylacetate.—A mixture of 1 g of diethylaminoethyl 2-(3-hydroxyphenyl)phenylacetate, 5 ml of Ac_2O, and 1 drop of pyridine was heated on a water bath for 2 hr. Excess Ac_2O was evapd and the residue was extd (Et_2O). The extract was washed (H_2O), dried (Na_2SO_4), and evapd. The remaining residue was chromatographed on silica gel to give 0.7 g (62.5%) of the acetate as a pale yellowish oil.

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Inhibition of Leucine Aminopeptidase by Halide Complexes of Platinum

ROBERT W. GUTHRIE, PAUL MELIUS,* AND JOHN E. TEGGINS*

Department of Chemistry, Auburn University, Auburn, Alabama 36830

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Leucine aminopeptidase levels have been found to be increased in tumors.[1] Recently Rosenberg,[2] *et al.*, reported that some halide complexes of Pt inhibited sarcoma 180 and leukemia L1210 in mice. In order to find a chemical basis for the sarcoma 180, leukemia L1210 inhibition, we have tested some Pt–halide complexes for inhibitory action on purified swine leucine aminopeptidase[3] (LAP).

LAP is a metal-requiring enzyme which has been shown to be active in the Mg^{2+} or Mn^{2+} form and more recently in the Zn^{2+} form by Himmelhoch.[4] The enzyme catalyzes stepwise hydrolysis from the N-terminal end of a polypeptide chain liberating free amino acids.

In the enzyme assay procedure with KBr added in the same concentration as the $PtBr_2$ complexes, no inhibition was observed in 50 hr at 37°. The 5×10^{-3} *M* tetrabromo complex of Pt inhibited completely the LAP within 1 hr. The ethylenediamine dibromo complex, $Pt(En)Br_2$, of Pt (5×10^{-3} *M*) resulted in over 80% inhibition of the LAP in 50 hr. The diethylenetriamine monobromo, $Pt(Dien)Br^{1+}$ complex, of (5×10^{-3} *M*) resulted in about 20% inhibition of LAP in 50 hr.

Rosenberg *et al.*,[2] found that the most active antitumor compounds were *cis*-$Pt(NH_3)_2Cl_4$; *cis*-$Pt(NH_3)_2Cl_2$; $Pt(NH_2CH_2CH_2NH_2)Cl_2$ and $Pt(NH_2CH_2CH_2NH_2)Cl_4$. Spikes and Hodgson[5] also found the $PdCl_2$ inhibited chymotrypsin and trypsin, but did not inhibit catalase, lysozyme, peroxidase, and ribonuclease at 1×10^{-3} *M* Pd^{2+} concentrations.

The structures of the Pt complexes employed in this study are illustrated in Figure 1. It has been frequently observed that halide ligands are more labile than amine ligands in substitution reactions of Pt(II) complexes. In fact many workers have utilized this effect in order to study substitution reactions of halide ligands. For example, Gray[6] has reported the results of substitution studies for a single halide ligand in Pt complexes in which the other 3 coordination positions were blocked by the tribasic amine, diethylenetriamine. It is very noticeable that the rates of inhibition of LAP by the 3 platinum complexes used in this study increase with an increasing number of halide ligands. As can be seen in Figure 2, $PtBr_4^{2-}$ completely deactivates the enzyme much more rapidly than $Pt(En)Br_2$. Pt-

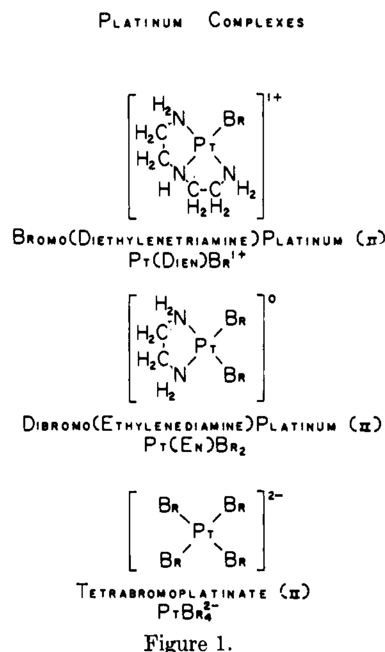

Figure 1.

* To whom correspondence should be addressed.

(1) Jean DeBersaques, *Arch. Klin. Exp. Dermatol.*, **234**, 5260 (1969).
(2) B. Rosenberg, L. Van Camp, J. E. Trosko, and V. H. Mansour, *Nature (London)*, **222**, 385 (1969).
(3) M. M. Moseley and P. Melius, *Can. J. Biochem.*, **45**, 1641 (1967).
(4) S. R. Himmelhoch, *Arch. Biochem. Biophys.*, **134**, 597 (1969).
(5) J. D. Spikes and C. F. Hodgson, *Biochem. Biophys. Res. Commun.*, **35**, 420 (1969).
(6) H. B. Gray, *J. Amer. Chem. Soc.*, **84**, 154 (1962).

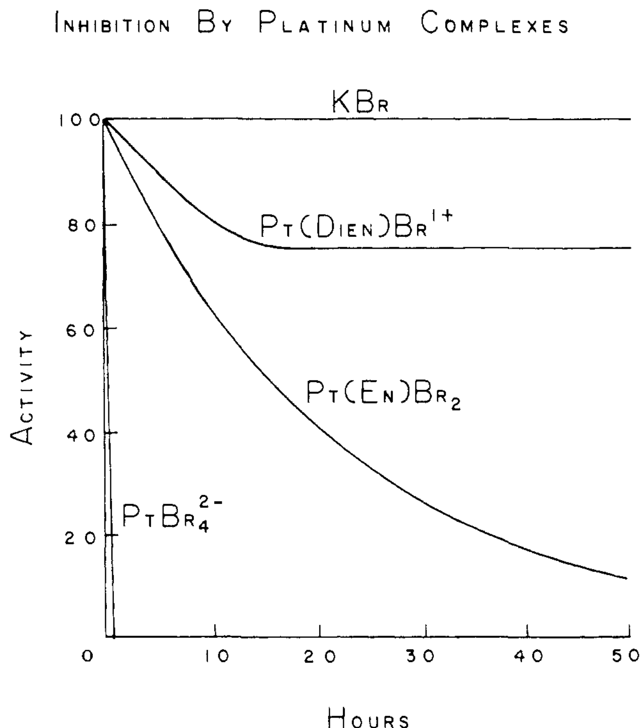

Figure 2.—Inhibition of Pt complexes. Four flasks containing 0.05 M $MgCl_2$, 0.025 M barbital buffer, pH 8.0, 20 μl of enzyme and the Pt complex or KBr standard, were placed in 37° water bath and aliquots were removed at various times and added to the assay vessel containing 0.05 M $MgCl_2$, 0.025 M barbital buffer, pH 8.0, and 0.01 M leucinamide.

(Dien)Br^+, which only contains a single Br^- ligand, did not deactivate the enzyme completely under the experimental conditions. It is well known that bidentate ligands, which are bound to the metal through two nucleophilic sites, are much more stable than comparable monodentate ligands. Thus, ethylenediamine complexes of Cu(II) have considerably larger formation constants than NH_3 complexes.[7] It is possible, therefore, that only Pt complexes which have at least 2 replaceable groups can be bound strongly to the enzyme with resultant deactivation. Nonbiological reactions in which a molecule containing cis nucleophilic groups displaces 2 halide ligands in a Pt(II) complex to produce a stable product have been reported.[8] It is also noticeable that all of the Pt complexes which have been shown to possess tumor-inhibiting properties by Rosenberg, *et al.*,[2] contain labile cis dihalide ligands. Mechanistic similarities between the enzyme-inhibition, tumor-inhibition, and inorganic substitution reactions are quite possible.

Experimental Section

Rubidium Tetrabromoplatinate(II).—K_2PtBr_6 was prepared by dissolving Pt wire in aqua regia, evaporating the resultant soln almost to dryness after the addition of excess HBr, and precipitating the hexabromoplatinate (IV) ion in the form of its K salt by the addition of sufficient K_2CO_3 to neutralize the remaining acid. A soln of K_2PtBr_4 was obtained by reduction of K_2PtBr_6 with an equivalent quantity of an aq oxalic acid. The sparingly sol Rb_2PtBr_4 was pptd from this soln by addn of excess RuBr. *Anal.*[9] ($Rb_2PtBr_4.H_2O$): Rb 24.3, Pt 27.7. Found: Rb 24.7; Pt 27.7.

Dibromo(ethylenediamine)platinum(II).—Pt(En)Br_2, was prepared by the direct reaction of equiv amounts of $Rb_2PtBr_4 \cdot H_2O$ and En in aq solution at pH 9. The experimentally determined Pt content of the complex (46.7%) was in good agreement with the theoretical value (47.0%).

Bromo(diethylenetriamine)platinum(II) bromide, [Pt(Dien)-Br]Br, was prepared and analyzed as described in a previous publication.[10]

Leucine Aminopeptidase.—The method of Moseley and Melius[3] was employed to prepare aq enzyme soln. Protein contents of enzyme prepns were estimated by the colorimetric procedure of Miller.[11] Assays of enzyme activity were performed by titrating NH_3 liberated from the hydrolysis of L-leucinamide using a Radiometer titrator, Type III 1e with a Titrigraph, Type SBR2C and syringe buret as a pH-Stat. All assays were carried out at pH 8.0 and 37°. A unit of enzyme activity hydrolyzed 1 equiv of L-leucinamide/min at a 0.01 M substrate concn.

(7) L. A. Sillen, Ed., "Stability Constants", Chemical Society, London, 2nd ed, 1964.
(8) J. V. Rund and F. A. Palocsay, *Inorg. Chem.*, **8**, 524 (1969).
(9) Analytical procedures: E. D. Smith, J. A. McCann, and J. E. Teggins, *ibid.*, **8**, 1872 (1969).
(10) J. E. Teggins and D. S. Martin, Jr., *ibid.*, **6**, 1003 (1967).
(11) G. Miller, *Anal. Chem.*, **31**, 964 (1959).

Some Pyrrolidine Derivatives as Antispasmodics

JOHN K. SUGDEN* AND MOHINDER SINGH

School of Pharmacy, City of Leicester Polytechnic, Leicester, England

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Potent analgetic activity has been reported in N-substituted-4-aminopiperidine derivatives,[1-3] in which the basic heterocyclic N is separated from an acylated anilino group by 3 C atoms. It was considered pertinent to prepare a series of N-(substituted phenyl)-N'-(1-phenethyl-3-pyrrolidinyl)acetamides (**1**) having this structural feature.

The amides 1 were prepared by a 4-stage synthesis (Scheme I) described in the Experimental Section. None of the compounds examined showed any analgetic activity, but they had potent antispasmodic activity in the Konzett–Rossler test.[4] Fifteen compounds of type 1 were tested against AcCh, histamine, and 5-HT in anesthetized guinea pigs; the results are presented in Table I.

Potent activity is found in compounds where R_1 is an unsubstituted aromatic ring. Substitution of the aromatic ring reduced the antispasmodic activity, electron-donating groups producing a less marked reduction in activity than electron-withdrawing groups. The nature of the alkyl group R_2 influenced the toxicity as well as the activity of the compounds. When R_2 was Me, activity was maximal and toxicity minimal; the reverse was true when R_2 was Et. The other groups tested at R_2 showed intermediate levels of toxicity and activity.

The phenethyl side chain was selected in view of the associations with potent analgetic activity.[3] All the compounds 1 showed significantly greater activity

(1) P. A. J. Janssen, British Patent No. 917,078 (1961).
(2) P. A. J. Janssen, *Brit. J. Anaesth.*, **34**, 260 (1962).
(3) N. J. Harper and C. F. Chignell, *J. Med. Chem.*, **7**, 729 (1964).
(4) H. Konzett and R. Rossler, *Arch. Exp. Path. Pharmacol.*, **195**, 71 (1940).