(10) W. Gilbert and B. Müller-Hill, *Proc. Natl. Acad. Sci. U.S.A.*, **58**, 2415 (1967).
(11) A. M. Sarquis and R. A. Zingaro, *J. Heterocycl. Chem.*, **10**, 909 (1973).
(12) H. King and R. J. Ludford, *J. Chem. Soc.*, 2086 (1950).
(13) K. B. Jacobson, J. B. Murphy and B. Das Sarma, *FEBS Lett.*, **22**, 80 (1972).
(14) G. P. Van der Kelen, *Bull. Soc. Chim. Belg.*, **65**, 343 (1956).
(15) I. C. Gunsalus, L. S. Barton, and W. Gruber, *J. Am. Chem. Soc.*, **78**, 1763 (1956).
(16) L. C. King, R. M. Doson, and L. A. Subluskey, *J. Am. Chem. Soc.*, **70**, 1176 (1948).
(17) M. Akagi, S. Tejima, and M. Haga, *Chem. Pharm. Bull.*, **11**, 559 (1963).

Platinum and Palladium Derivatives for Chemotherapy Studies[1]

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Analogues of *cis*-dichlorodiammineplatinum(II) were prepared in which substituted pyridines (A), 1-(4-aminobenzylidene)indene (B), or DL-3,5,3′5′-tetraoxo-1,2-dipiperazinopropane (ICRF-159) was used in place of ammonia, and in some cases platinum(IV) or palladium(II) was used in place of the platinum(II). Both platinum complexes with ICRF-159 were active against leukemia 1210, but none of the others produced significant life extension following a single ip dose of 400 mg/kg. Attempts to prepare complexes of ICRF-159 with Zn(II), Mn(II), and Cr(III) were unsuccessful, but there were indications of complex formation with $CuCl_2$ and with $NiCl_2$.

The announcement by Rosenberg et al.[2] that *cis*-dichlorodiammineplatinum(II) had antitumor activity encouraged us to prepare analogues containing other nitrogen compounds and submit them for testing. The first samples we submitted were *cis*-dichlorobis(substituted pyridine)platinum(II) compounds whose preparation and physical properties had been reported by Patterson and Bull.[3] None of these was effective against L-1210 leukemia at a single dose of 400 mg/kg, but some were toxic (see Table I). None of them has been tested by the methods used by Gale, Howle, and Walker,[4] who found *cis*-dichlorobis(pyridine)platinum(II) effective in several in vitro systems and against Ehrlich ascites carcinoma in vivo.

1-(4-Aminobenzylidene)indene (1) has been found to exert a strong effect against the growth of Walker 256 tumors in rats,[5] but not against L-1210 in mice. It formed palladium and platinum complexes which were inactive against L-1210 at 400 mg/kg and did not kill any of the animals at that dose level.

DL-3,5,3′,5′-Tetraoxo-1,2-dipiperazinopropane (ICRF-159) has attracted attention because of activity against several tumors. Woodman et al.[6] reported a marked synergism when ICRF-159 and *cis*-dichlorodiammineplatinum(II) were administered to BDF_1 mice after ic innoculation with L-1210. It seemed possible that the administration of a compound containing both ICRF-159 and platinum(II) might be more effective than separate administration of two drugs. Complexes of ICRF-159 with divalent and tetravalanet platinum and divalent palladium were prepared.

Creighton[7] found that the (ICRF-159)$PtCl_2$ complex inhibited [^{3}H]thymidine uptake in vitro to about the same extent as ICRF-159 itself, on a molar basis (see Table II). In tests against L-1210 under the auspices of the National Cancer Institute it produced life extension: 9 × 200 mg/kg doses gave T/C = 1.21 and 9 × 400 mg/kg gave T/C = 1.60. The tetrachloro(ICRF-159)platinum(IV) complex was active against L-1210 at a lower dose level than the platinum(II) complex: 3 × 100 mg/kg gave T/C = 1.43 and 9 × 12.5 mg/kg gave T/C = 1.33.

Experimental Section

Dichlorobis[1-(4-aminobenzylidene)indene]platinum(II). To a stirred solution of 220 mg (1.0 mmol) of 1 in 15 mL of tetrahydrofuran (THF) and 5 mL of H_2O was added 209 mg (0.5 mmol) of K_2PtCl_4. The solution was held at 60 °C in a H_2O bath for 1 h and then allowed to stand overnight. The yellow precipitate which began to appear soon after the mixing was washed with H_2O and THF, yield 169 mg (48%). Anal. ($C_{32}H_{26}N_2PtCl_2$) C, H.

Dichlorobis[1-(4-aminobenzylidene)indene]palladium(II). A solution of 219 mg (1.0 mmol) of 1 in 10 mL of THF and 5 mL of H_2O was added slowly to a solution of 164 mg (0.5 mmol) of K_2PdCl_4 in 12.5 mL of H_2O and 12.5 mL of THF. After 2 h, the orange precipitate was washed successively with H_2O, MeOH, C_6H_6, and Et_2O and then dried, yield 297 mg (97%). Anal. ($C_{32}H_{26}N_2PdCl_2$) C, H, N.

Dichloro(ICRF-159)palladium(II). A solution of 4.5 mmol of ICRF-159 in 450 mL of H_2O was added, during 2–3 min, to a solution of 4.8 mmol of K_2PdCl_4 in 20 mL of H_2O. After stirring the mixture 5 h, it was filtered. The yellow precipitate was washed with two 3-mL portions of MeOH and then dried, yield 87%. Anal. ($C_{11}H_{16}N_4O_4PdCl_2$) C, H, Pd. In another run, the ICRF was dissolved in THF and the K_2PdCl_4 in 80:20 THF–H_2O before mixing. A small amount of black precipitate was removed by filtration, and then the filtrate was evaporated under reduced pressure, producing a yellow precipitate.

Dichloro(ICRF-159)platinum(II). Approximately 9.0 mmol of Na_2PtCl_4 dissolved in 1 L of acetone was added during 20 min to 4.5 mmol of ICRF-159 dissolved in 1.2 L of boiling acetone. After the solution was left standing overnight at room temperature, the tan precipitate was recovered. More was obtained by evaporation of the acetone under reduced pressure. The product was washed twice with 2-mL portions of H_2O and twice with 1-mL portions of MeOH and then dried: yield 75%; mp 275–289 °C. Anal. ($C_{11}H_{16}N_4O_4PtCl_2$) C; H: calcd, 24.73; found, 24.30. Another sample prepared in H_2O solution gave C: calcd, 24.94.

Tetrachloro(ICRF-159)platinum(IV). A solution of 3.5 mmol of ICRF-159 in 800 mL of acetone was added to a solution of 3.3 mmol of $Na_2PtCl_6·6H_2O$ in 185 mL of acetone at room temperature during 15 min. After 2 days a white precipitate was filtered off and discarded. The solution was evaporated under vacuum to dryness. The resulting yellow precipitate was washed with 50 mL of THF and three times with 2–3 mL of MeOH and then dried: yield 98%; mp 240–250 °C dec. Anal. ($C_{11}H_{16}N_4O_4PtCl_4$) C, H, Pt.

Attempts to prepare complexes of ICRF-159 with Zn(II), Mn(II), and Cr(III) did not meet with any success, but what appeared to be an 80% yield of an impure green complex of $CuCl_2$ with ICRF-159 was obtained when a solution of 270 mg of ICRF-159 in 150 mL of hot absolute EtOH was added to a solution of 170 mg of $CuCl_2·2H_2O$ in 25 mL of absolute EtOH. The mixture was stirred for 1 h, cooled, and filtered. Water appeared to decompose the substance, and no satisfactory solvent for recrystallization was found. Likewise, a green solid was obtained by mixing 140 mg of ICRF-159 dissolved in 50 mL of DMF with a solution of 120 mg of $NiCl_2·6H_2O$ in 50 mL of DMF, allowing the mixture to stand overnight, removing DMF under vacuum,

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Table I. Antileukemic Activity[a]

Pt and Pd deriv	no. of inject.	day of eval	dose, mg/kg	no. of surv	no. of treat. anim	control body wt change, g	anim wt diff (T − C), g	tumor eval test control, days surv		% T/C
cis-dichlorobis-										
(3-bromopyridine)Pt	1	30	400	6	6	0.4	0.9	8.5	9.1	93
(4-isopropylpyridine)Pt	1	30	400	1	6	0.4	−3.7	1.0	9.1	
(4-ethylpyridine)Pt[b]	1	30	400	3	6	0.4	−1.0	8.7	9.1	
(4-bromopyridine)Pt	1	30	400	1	6	0.4	−2.9	6.0	9.1	
(4-chloropyridine)Pt[b]	1	30	400	6	6	0.4	0.7	9.8	9.1	107
(α-inden-1-ylidene-*p*-toluidine)Pt	1	30	400	6	6	0.6	−1.0	8.3	9.5	87
(isoniconitonitrile)Pt[b]	1	30	400	6	6	0.4	0.0	9.7	9.1	106
	3	30	400	0	6	2.0	−2.0	0.0	8.8	
	3	30	200	3	6	2.0	−2.2	8.7	8.8	
	3	30	100	6	6	2.0	−1.0	8.8	8.8	100
(nicotinonitrile)Pt[b]	1	30	400	1	6	0.4	0.8	10.0	9.1	
	3	30	400	2	6	2.0	−0.8	8.0	8.8	
	3	30	200	6	6	2.0	−6.3	8.2	8.8	93
	3	30	100	6	6	2.0	−2.5	7.2	8.8	
(ICRF-159)Pt	3	30	100	6	6	1.0	−0.9	11.1	10.3	107
	3	30	50	6	6	1.0	−0.6	11.3	10.3	109
	3	30	25	6	6	1.0	1.2	10.0	10.3	97
	3	30	12.5	6	6	1.0	0.6	11.0	10.3	106
	9	30	100	6	6	1.0	−1.1	10.3	9.5	108
	9	30	50	6	6	1.0	−0.7	10.8	9.5	113
	9	30	25	6	6	1.0	−0.4	10.1	9.5	106
	9	30	12.5	6	6	1.0	−0.7	10.0	9.5	105
	3	30	400	6	6	2.0	−0.6	11.8	10.0	118
	3	30	200	6	6	2.0	−0.2	10.1	10.0	101
	3	30	100	6	6	2.0	−0.4	10.6	10.0	106
	9	30	400	6	6	1.0	−2.1	15.2	9.5	160
	9	30	200	6	6	1.0	−0.6	11.5	9.5	121
	9	30	100	6	6	1.0	−0.2	10.3	9.5	108
tetrachlorobis(ICRF-159)Pt	3	30	400	0	6	−0.4	0.4	0	9.9	
	3	30	200	0	6	−0.4	0.4	0	9.9	
	3	30	100	6	6	−0.4	−1.5	15.2	9.9	153
	3	30	50	6	6	−0.4	−0.1	10.8	9.9	109
	3	30	150	4	6	1.6	−4.2	10.2	9.0	116
	3	30	100	5	6	1.6	−1.7	12.0	9.0	133
	3	30	50	6	6	1.6	−2.1	10.8	9.0	120
	3	30	25	6	6	1.6	−0.8	9.8	9.0	108
	9	30	100	6	6	−0.3	0.2	10.8	9.4	114
	9	30	50	6	6	−0.3	0.1	11.3	9.4	120
	9	30	25	6	6	−0.3	0.5	10.8	9.4	114
	9	30	12.5	4	6	−0.3	−1.6	14.3	9.4	152
	9	30	200	0	6	0.8	−0.8	0	10.2	
	9	30	100	1	6	0.8	−5.9	6.0	10.2	
	9	30	50	6	6	0.8	−2.7	14.0	10.2	137
	9	30	25	6	6	0.8	−1.7	12.3	10.2	120
	9	30	12.5	6	6	0.8	−0.8	11.8	10.2	115
	9	30	6.25	6	6	0.8	0.8	11.3	10.2	110
	9[c]	30	400	6	6	−2.4	2.7	19.8	10.8	183
	9[c]	30	200	6	6	−2.4	2.6	15.3	10.8	141
	9[c]	30	100	6	6	−2.4	2.4	16.0	10.8	148
	9[c]	30	50	6	6	−2.4	3.1	14.0	10.8	129
	9[c]	30	25	6	6	−2.4	2.7	12.8	12.8	118
	9	30	12.5	6	6	−2.4	2.9	10.8	10.8	100
	9[c]	20	400	6	6	1.2	0.0	14.0	12.3	113
	9[c]	20	200	6	6	1.2	0.1	13.0	12.3	105
	9[c]	20	100	6	6	1.2	0.2	11.3	12.3	91
	9[c]	20	50	6	6	1.2	0.8	12.0	12.3	97
	9	20	25	6	6	1.2	−3.8	11.8	12.3	95
dichloro[4,4′-	2	30	400	3	3	1.4	−1.7	9.0	9.5	94
(1-methyl-1,2-ethanediyl)-	2	30	200	3	3	1.4	0.0	10.7	9.5	112
bis(2,6-piperazinedione)]Pd	2	30	100	3	3	1.4	−0.4	10.3	9.5	108
cis-dichlorobis(α-inden-1-	1	30	400	6	6	0.6	−1.6	9.0	9.5	94
ylidene-*p*-toluidine)Pd										

[a] Leukemic 1210 system unless otherwise indicated. For general screening procedure and data interpretation, cf. R. I. Geran, N. H. Greenberg, H. M. MacDonald, A. M. Schumann, and B. J. Abbott, *Can. Chem. Rep.*, 3(2), 1 (1972); Instruction Booklet 14, "Screening Data Summary Interpretation", Drug Research and Development, Chemotherapy, National Cancer Institute, Bethesda, Md., 1972. A single 8 mg/kg dose of *cis*-dichlorodiammineplatinum(II) produced a 380% increase in life span of animals in the Leukemia 1210 test. Rosenberg and Barrett, *Platinum Metals Rev.*, 15(2), 3-12 (1971). [b] Cf. S. J. Meischen, G. R. Gale, L. M. Lake, C. J. Frangakis, M. G. Rosenblum, E. M. Walker, Jr., L. M. Atkins, and A. B. Smith, *J. Natl. Cancer Inst.*, 57, 841-845 (1976). [c] PS leukemia system.

Table II. Effect of 24-h Treatment on Uptake of [^{3}H]Thymidine by Secondary Mouse Embryo Fibroblast Cultures[a]

compd	concn, mg/mL	change, %
$PtCl_4$-ICRF-159	10.0	−71
	1.0	−16
	0.1	−8
$PtCl_2$-ICRF-159	10.0	−79
	1.0	−30
	0.1	−7
$PdCl_2$-ICRF-159	10.0	−17
	1.0	−2
	0.1	+21
ICRF-159	10.0	−73
	1.0	−57
	0.1	−20

[a] A. M. Creighton, Imperial Cancer Research Fund, private communication; for method, see A. M. Creighton and G. D. Birnie, *Int. J. Cancer*, 5, 47–54 (1970).

and washing the oily yellow residue with THF: yield 75%; mp 280–290 °C dec. The visible absorption spectrum indicated that a chemical change did take place, but the product was not purified further.

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References and Notes

(1) Presented at the Southeastern Regional Meeting of the American Chemical Society, Charleston, S.C., Nov., 1973.
(2) B. Rosenberg, L. Van Camp, J. E. Trosko, and V. H. Mansour, *Nature (London)*, **222**, 385 (1969).
(3) T. C. Patterson, "Synthesis of Some *cis*-Dichlorobis(substituted pyridine)platinum(II) Compounds and Studies of the Chloride Exchange, Solvolysis, and 2,2′-Bipyridine Reactions of Some *cis*-Dichlorobis(substituted pyridine)-platinum(II) Compounds in Dimethylsulfoxide", 1966, unpublished doctoral dissertation.
(4) G. R. Gale, J. A. Howle, and E. M. Walker, Jr., *Cancer Res.*, **31**, 950 (1971).
(5) C. T. Bahner, H. Kinder, D. Brotherton, J. Spiggle, and L. Gutman, *J. Med. Chem.*, 8, 390 (1965).
(6) R. J. Woodman, J. M. Venditti, S. A. Schepartz, and I. Kline, *Proc. Am. Assoc. Cancer Res.*, **12**, 24 (1971).
(7) Creighton, A. M., private communication.

Synthesis of All the Stereoisomers of Statine (4-Amino-3-hydroxy-6-methylheptanoic Acid). Inhibition of Pepsin Activity by *N*-Carbobenzoxy-L-valyl-L-valyl-statine Derived from the Four Stereoisomers

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Synthesis of all four stereoisomers of the novel amino acid statine, 4-amino-3-hydroxy-6-methylheptanoic acid, found in pepstatin, a potent acid protease inhibitor, has been accomplished. Carbobenzoxy-L-valyl-L-valyl-statine tripeptides derived from all four stereoisomers have been prepared and their effect on pepsin activity is compared to that of pepstatin.

Pepstatin, isovaleryl-L-valyl-L-valyl-(3*S*,4*S*)-statyl-L-alanyl-(3*S*,4*S*)-statine,[1] is one of several peptide antibiotics isolated in a deliberate attempt to find low-molecular-weight protease inhibitors that were amenable to chemical synthesis.[2] This peptide inhibits renin, pepsin, and cathepsin D activities and shows promise in the treatment of ulcers, inflammation and hypertension,[2] and, recently, muscular dystrophy.[3,4] While pepstatin is a very effective inhibitor, it is not very selective, which may be a contributing factor as to why it is not in clinical use. Thus, synthesis of analogues and derivatives that might be more selective is warranted. The development of a convenient synthetic route to the novel amino acid statine, (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid (**3**), and analogues was the initial objective of the research. While several syntheses of statine have been reported,[5-9] a more convenient method for separation of the (3*S*,4*S*) and (3*R*,4*S*) diastereomers is outlined in this paper. The difficulty of separating the diastereomers was solved by chromatographing them on commercial Lobar silica gel columns as did Rich et al.[9] In this note, the preparation of all four stereoisomers of statine, the synthesis of the four statine *tert*-butyl esters and their use in the preparation of Cbz-L-valyl-L-valyl-statine tripeptides, and a comparison of the tripeptides and pepstatin as inhibitors of pepsin activity are reported.

Chemistry. Synthesis of statine (**3**) is outline in Scheme I. The mixtures of diastereomers, **2a,b** and **2c,d**, were prepared by condensation of the appropriate phthalyl-leucinal with the zinc enolate of *tert*-butyl acetate.[8] Each diastereomeric mixture was resolved into its pure components by preparative liquid chromatography for a combined yield of 57%. The use of Boc and ethyl ester blocking groups appears to make the separation easier;[9] the increased bulk of the blocking groups used in the work reported here offers greater steric hindrance to the interaction of the polar groups with the silica gel. The free amino acids, **3**, were obtained in 94% yield from **2** by removal of the *tert*-butyl ester with CF_3COOH and the phthalyl group by hydrazinolysis. Physical data for statine stereoisomers are given in Table I. The melting points of **3a** and **3c** differed from those of **3b** and **3d** as expected of diastereomers, but all of our melting points were higher than those reported.[5-7] The differences could in part be due to variations in thermometers, heating rates, or the temperature at which the samples were introduced during the melting point determinations and to the greater purity achieved by high-pressure liquid chromatography than by methods used in previous work. The optical rotations of **3a–d** are in reasonable agreement with reported[6] values. The tripeptide *tert*-butyl esters were prepared (Scheme II) from *tert*-butyl esters, **4**, which were obtained in 68%

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