Some Nuclear-Substituted Derivatives of α-Methyldopa[1]

ANILKUMAR PARULKAR, ALFRED BURGER,

Department of Chemistry, University of Virginia, Charlottesville, Virginia 22903

AND DOROTHEA AURES

Psychopharmacology Research Laboratories, Veterans Administration Hospital, Sepulveda, California 91343

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The synthesis of 2- and 6-methyl, 2-chloro, and 2-fluoro derivatives of α-methyldopa has been performed. These compounds have been tested as inhibitors of dopa decarboxylase, tyrosine hydroxylase, and of various viruses *in vitro*. The 2-chloro analog is a better hog kidney dopa decarboxylase inhibitor than α-methyldopa.

Derivatives of dopa have been tested for various biological activities, notably as inhibitors of dopa decarboxylases with a sequel in the control of elevated blood pressure,[2] as virustatic agents,[3,4] etc. We were interested in exploring the composite effect of steric hindrance, both in the benzene nucleus and in the side chain, on the prolongation or intensification of some of these activities which may result from the protection of side-chain degradation. Therefore, 2- and 6-methyl-, 2-chloro-, and 2-fluoro-substituted derivatives of α-methyldopa were synthesized, essentially by the method used by Stein, *et al.*,[5] in the preparation of α-methyldopa, starting from the respective chloromethylveratroles. These steps, along with various modifications, are outlined in the Experimental Section.

Biological and Biochemical Tests.—2-Amino-2-methyl-3-(3,4-dihydroxy-2-tolyl)propionic acid (**28**), its 3,4-dimethyl ether (**22**), 2-amino-2-methyl-3-(4,5-dihydroxy-2-tolyl)propionic acid (**33**), its 4,5-dimethyl ether (**26**), 2-amino-2-methyl-3-(2-chloro-3,4-dihydroxyphenyl)propionic acid (**30**), and 2-amino-2-methyl-3-(2-fluoro-3,4-dihydroxyphenyl)propionic acid (**32**) were tested in several *in vitro* systems.

Dopa decarboxylase inhibition tests were carried out with hog kidney dry powder under conditions described in the Experimental Section. The results, compiled in Table I, show that **30** is a better inhibitor than α-methyldopa.

TABLE I

INHIBITION OF HOG KIDNEY DOPA DECARBOXYLASE BY ANALOGS OF α-METHYLDOPA

Compd	% inhib, M					Molarity at 50% inhib
	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	
DL-α-Methyldopa	100	95	70	30	21	7×10^{-6}
30	100	82	65	50	20	10^{-6}
32	100	78	40	22	20	4×10^{-5}
28	34	32	30	...	...	...
33	20	12	10	...	...	...

Tyrosine hydroxylase inhibition tests were carried out by Dr. Edith G. McGeer; the methodology is described in the Experimental Section. Only DL-2-fluoro-α-methyldopa (**32**) caused an inhibition of 24%.

Antiviral tests, carried out by Dr. E. Furasawa, showed that at the maximum nontoxic doses **28** (25 μg/ml), **33** (12 μg/ml), and **30** (25 μg/ml) failed to show inhibitory activity against Columbia SK virus (RNA type) in Ehrlich ascites tumor cells,[6] and at 5 μg/ml against LCM virus (RNA type), Vaccina (IHD, neurotropic strain) (DNA type), and Adeno virus type 12 (DNA type) in KB cell tissue culture.

Experimental Section[7]

Substituted Chloromethylveratroles.—Following a study by Vavon, *et al.*,[8] concerning reaction rates of the chloromethylation with chloromethyl ether, the chloromethylation of 3-methylveratrole was performed at 34–35° for 7 hr, yielding 64% of 2-methyl-3,4-dimethoxybenzyl chloride as compared with 42% found previously. In a similar fashion, 3-chloro-[9] and 3-fluoro-4-chloromethylveratrole[3] were prepared.

4-Chloromethyl-5-methylveratrole.—A solution of 16.0 g (0.105 mole) of 4-methylveratrole and 16.8 g (0.21 mole) of chloromethyl ether in 16.0 g of glacial acetic acid was warmed at 34–36° with stirring for 6 hr. The mixture was quenched with 200 ml of ice-water, the separated oil was extracted three times with ether, and the ether solution was washed (10% $NaHCO_3$, H_2O), dried (Na_2SO_4), and evaporated. A fraction of the residual oil boiling at 93° (0.1 mm) (8.0 g, 40%) showed a correct analysis (Table II) for the desired chloromethyl compound and exhibited the expected infrared spectrum. The position of the chloromethyl group was established by oxidation of the compound with aqueous $KMnO_4$ to 6-methylveratric acid, mp 145–146°,[10] as well as by converting it to 6-methylveratraldehyde, mp 70–71°,[11,12] bp 94–98° (0.05 mm),[12] by heating with dimethyl sulfoxide in the presence of $NaHCO_3$ as an acid acceptor.[13]

After 4-chloromethyl-5-methylveratrole had distilled, a higher boiling fraction which solidified in the receiver was collected. It was recrystallized from ethanol with the aid of charcoal, mp 121.5–122°.

(1) Supported by Grants NB-1445, MH-03663, and GM-001882 from the National Institutes of Health, U. S. Public Health Service.

(2) For a comprehensive review, see A. R. Patel and A. Burger, *Progr. Drug Res.*, **9**, 223 (1966).

(3) C. Kaiser and A. Burger, *J. Am. Chem. Soc.*, **79**, 4365 (1957).

(4) A. Burger, W. E. Coyne, and G. Janssen, *J. Med. Chem.*, **6**, 614 (1963).

(5) G. A. Stein, H. A. Bronner, and K. Pfister, 3rd, *J. Am. Chem. Soc.*, **77**, 700 (1955).

(6) E. Furasawa, W. Cutting, and A. Furst, *Chemotherapia*, **8**, 94 (1964).

(7) Analyses and physical data are listed in Table II. Melting points were determined on a Fisher-Johns melting point apparatus and are corrected. Infrared spectra were read on a Perkin-Elmer Model 337 spectrophotometer, using KBr pellets, or pure liquids, respectively. Nmr spectra were measured with a Varian Associates A-60 spectrometer, with tetramethylsilane as an internal reference standard. Analyses were performed by Geller Microanalytical Laboratories, Charleston, W. Va., and Galbraith Laboratories, Inc., Knoxville, Tenn.

(8) G. Vavon, J. Bolle, and J. Calin, *Bull. Soc. Chim. France*, [5] **6**, 1025 (1939).

(9) E. D. Hornbaker and A. Burger, *J. Am. Chem. Soc.*, **77**, 5314 (1955).

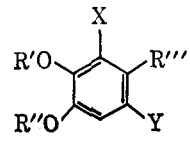
(10) A. St. Pfau, *Helv. Chim. Acta*, **22**, 550 (1939), reported mp 146–147°.

(11) L. Gatterman, *Ann.*, **357**, 313 (1907), gave mp 75°.

(12) J. M. Bruce and F. K. Sutcliffe, *J. Chem. Soc.*, 3824 (1956), found mp 74°, bp 110–111° (0.2 mm).

(13) H. R. Nace and J. J. Monagle, *J. Org. Chem.*, **24**, 1792 (1959).

TABLE II



No.[a]	X	Y	R'''	Yield, %	Bp (mm) or mp, °C	Crystn solvent[b]	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N
1		CH_3	CH_2Cl	40	93 (0.1)	C–P	$C_{10}H_{13}ClO_2$	59.85	6.52		59.56	6.42	
2	CH_3	H	CH_2CN	83	66–66.5	C–P	$C_{11}H_{13}ClO_2$	69.08	6.85		68.86	6.65	
3	Cl	H	CH_2CN	76	65–65.5	C–P	$C_{10}H_{16}ClNO_2$	57.21	4.76		57.24	4.53	
4	F	H	CH_2CN	60	53–53.2	C–P	$C_{10}H_{10}FNO_2$	61.17	5.16		61.23	5.20	
5	H	CH_3	CH_2CN	75	94.5–95		$C_{11}H_{13}NO_2$	69.08	6.85		69.32	6.85	
6	CH_3	H	$CH(CN)COCH_3$	76	128 (0.1)		$C_{13}H_{15}NO_3$	66.93	6.48		67.12	6.45	
7	Cl	H	$CH(CN)COCH_3$	75	140 (0.2)		$C_{12}H_{12}ClNO_3$	56.80	4.76		57.02	4.89	
8	F	H	$CH(CN)COCH_3$	74	110 (0.05)		$C_{12}H_{12}FNO_3$	60.75	5.18		60.54	5.21	
9	H	CH_3	$CH(CN)COCH_3$	74	140 (0.05)		$C_{13}H_{15}NO_3$	66.93	6.48		67.13	6.60	
10	CH_3	H	CH_2COCH_3	63	95 (0.03)		$C_{12}H_{16}O_3$	69.20	7.74		69.02	7.90	
11	Cl	H	CH_2COCH_3	64	110 (0.1)		$C_{11}H_{13}ClO_3$	57.77	5.72		57.96	5.66	
12	F	H	CH_2COCH_3	40	95 (0.05)		$C_{11}H_{13}FO_3$	62.25	6.17		62.04	6.11	
13	H	CH_3	CH_2COCH_3	50	100 (0.05)		$C_{12}H_{16}O_3$	69.20	7.74		69.02	7.61	
14	CH_3	H	$CH_2C(CH_3)$=DNP[c]		141.3–141.5	E	$C_{18}H_{20}N_4O_6$	55.66	5.19		55.63	5.35	
15	Cl	H	$CH_2C(CH_3)$=DNP[c]		147.8–148.3	E	$C_{17}H_{18}ClN_4O_6$	49.96	4.19		49.78	4.10	
16	H	CH_3	$CH_2C(CH_3)$=DNP		125.3–127.3	E	$C_{18}H_{20}N_4O_6$	55.66	5.19		55.46	5.09	
17	F	H	$CH_2C(CH_3)$=DNP		157.5–158	E	$C_{17}H_{18}FN_4O_6$	52.03	4.36		51.99	4.35	
18	CH_3	H	$CH_2C(CH_3)CONHCONH$	92	218.5–219	E–W	$C_{14}H_{18}N_2O_4$	60.41	6.51		60.15	6.44	
19	Cl	H	$CH_2C(CH_3)CONHCONH$	95	215.5–216	E	$C_{13}H_{15}ClN_2O_4$	52.26	5.06		52.07	5.05	
20	F	H	$CH_2C(CH_3)CONHCONH$	83	202–202.5	E	$C_{13}H_{15}FN_2O_4$	55.31	5.35		55.03	5.19	
21	H	CH_3	$CH_2C(CH_3)CONHCONH$	67	235–235.5	E	$C_{14}H_{18}N_2O_4$	60.41	6.51		60.46	6.47	
22	CH_3	H	$CH_2C(CH_3)(NH_2)CO_2H$	90	262–263	W	$C_{13}H_{19}NO_4$	61.64	7.56	5.53	61.42	7.52	5.44
23	Cl	H	$CH_2C(CH_3)(NH_2)CO_2H$	92	271–272	W	$C_{12}H_{16}ClNO_4$	52.65	5.89	5.11	52.45	5.68	5.29
24	F	H	$CH_2C(CH_3)(NH_2)CO_2H$	91	282–283	W	$C_{12}H_{16}FNO_4$	56.02	6.27	5.45	56.09	6.31	5.63
25	F	H	$CH_2C(CH_3)(NH_3^+Cl^-)CO_2H$	100	234–235	E–AE	$C_{12}H_{17}ClFNO_4$	49.06	5.83	4.76	49.18	5.82	4.74
26	H	CH_3	$CH_2C(CH_3)(NH_2)CO_2H \cdot H_2O$	85	266–267	W	$C_{13}H_{21}NO_5$	57.54	7.80		58.09	7.89	
27	H	CH_3	$CH_2C(CH_3)(NH_3^+Cl^-)CO_2H$	100	234–235	E–AE	$C_{13}H_{20}ClNO_4$	53.88	6.95	4.85	53.70	7.07	4.61
28	CH_3	H	$CH_2C(CH_3)(NH_2)CO_2H$	95[d]	278[e]	[f]	$C_{11}H_{15}NO_4$	58.65	6.71	6.21	58.44	6.64	6.25
29	CH_3	H	$CH_2C(CH_3)(NH_3^+Cl^-)CO_2H$		267[e]		$C_{11}H_{16}ClNO_4$	50.47	6.16	5.35	50.53	6.21	5.25
30	Cl	H	$CH_2C(CH_3)(NH_2)CO_2H$	95[d]	254[e]	[f]	$C_{10}H_{12}ClNO_4$	48.88	4.92	5.70	48.62	4.83	5.88
31	Cl	H	$CH_2C(CH_3)(NH_2)CO_2H \cdot H_2O$		249[e]	[f]	$C_{10}H_{14}ClNO_5$	44.03	5.34		43.92	4.58	
32	F	H	$CH_2C(CH_3)(NH_2)CO_2H$	89[d]	281[e]	[f]	$C_{10}H_{12}FNO_4$	52.39	5.27	6.11	52.23	5.13	5.92
33	H	CH_3	$CH_2C(CH_3)(NH_2)CO_2H$	95[d]	247[e]	[f]	$C_{11}H_{15}NO_4$	58.65	6.71	6.21	58.44	6.95	6.14

[a] Compounds 1–**27**, R′ = R″ = CH_3; compounds **28–33**, R′ = R″ = H. All liquids were colorless, all solids were colorless crystals except 14–**16** which were orange crystals. [b] P = petroleum ether (bp 30–60°), C = $CHCl_3$, E = EtOH, AE = anhydrous ether, W = H_2O. [c] DNP = 2,4-dinitrophenylhydrazone. [d] Yields are those of crude reaction products; loss on purification, 15–20%. [e] Decomposition temperatures; all these compounds turned dark brown at 295–300°. [f] Analytical samples were prepared by dissolving the amino acid in the minimum amount of 20% HCl and adjusting the pH to 5.7–6 with a saturated solution of NaOAc. The crystals were filtered off, washed with cold water, and dried at 110° (0.025 mm, P_2O_5) for 24 hr.

Anal. Calcd for $C_{19}H_{24}O_4$: C, 72.12; H, 7.64; mol wt, 316. Found: C, 71.97; H, 7.63; mol wt (Rast), 305.

Since the chloromethylation of reactive phenols, aryl ethers, etc., often leads to diarylmethane or 9,10-dihydroanthracene derivatives,[14] the compound is apparently **di(4,5-dimethoxy-2-tolyl)methane.** The integrated nmr spectrum of this compound was in excellent agreement with this structure. Careful oxidation with SeO_2 at 120–130° without a solvent gave a brown viscous oil whose infrared spectrum contained a C=O absorption band.[15]

Substituted 3,4-Dimethoxyphenylacetonitriles.—To a suspension of NaCN (0.01 mole) in dimethyl sulfoxide (50 ml) warmed to 50°, the substituted benzyl halide (0.01 mole) was added in one portion with stirring. The exothermic reaction raised the temperature to 70–80°. After stirring and warming to 135–145° for 1–2.5 hr, the brown reaction mixture was cooled and stirred into crushed ice and water. With the exception of the 6-methyl isomer which separated out as a solid and could be filtered off, the oily nitriles were extracted into ether. The ether extracts were washed (H_2O), dried (Na_2SO_4), and distilled; the nitriles solidified in the receiver and appeared as large beautiful crystals

(14) R. C. Fuson and C. H. McKeever, *Org. Reactions*, **1**, 63 (1942).

(15) J. J. Postowsky and B. P. Lugowkin, *Ber.*, **68**, 852 (1935), prepared benzophenone by heating diphenylmethane with SeO_2 to 200-210° for 30 min. In the case of our compound, the reaction was vigorously exothermic above 145°.

after recrystallization. They absorbed strongly at 2255–2265 cm^{-1}.

Dimethyl sulfoxide was chosen as a solvent[16] after trials in ethanol[17] furnished ethyl 2-substituted 3,4-dimethoxybenzyl ethers as described in methods A and B.

A.—To a hot solution of 1.30 g (0.026 mole) of NaCN in 4.0 ml of water was added a solution of 5.015 g (0.025 mole) of 4-chloromethyl-3-methylveratrole in 20 ml of 95% ethanol over a period of 10 min. The mixture was refluxed for 6–8 hr, concentrated to one-half volume, and filtered. The filtrate was distilled and yielded 4.25 g (77%) of ethyl 3,4-dimethoxy-2-methylbenzyl ether as a colorless mobile oil, bp 98° (0.65 mm), 70° (0.025 mm). The integrated nmr spectrum was in good agreement with the proposed structure.

Anal. Calcd for $C_{12}H_{18}O_3$: C, 68.55; H, 8.65. Found: C, 69.03; H, 8.80.

B.—3-Chloro-4-chloromethylveratrole (18.0 g), treated similarly (see A), yielded two fractions; one of them consisted of 3.0 g of 2-chloro-3,4-dimethoxybenzyl ethyl ether as a colorless mobile liquid, bp 135–140° (0.85 mm).

Anal. Calcd for $C_{11}H_{15}ClO_3$: C, 57.26; H, 6.55. Found: C, 57.46; H, 6.56.

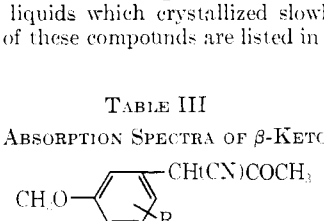
The second fraction appeared as 9.0 g of an oil, bp 144–153° (0.85 mm), which solidified in the receiver, and was identical with 2-chloro-3,4-dimethoxyphenylacetonitrile as obtained in dimethyl sulfoxide medium.

In dioxane, with or without catalytic amounts of NaI, unchanged starting material was recovered.

α-(3,4-Dimethoxyphenyl)acetylacetonitriles.—To a hot solution of sodium ethoxide prepared from 0.12 g-atom of Na in 40 ml of absolute ethanol was added a solution of 0.04 mole of the nitrile in 14.2 g (0.16 mole) of anhydrous ethyl acetate. After refluxing for 4 hr the mixture, which contained a heavy precipitate of the sodium salt, was allowed to stand at 25° for 14 hr, and the precipitate was filtered off, washed with anhydrous ethyl acetate and ether, and dried. A solution of the sodium salt in 60 ml of water was cooled and acidified with glacial acetic acid. After stirring for about 15 min the oil which had separated out was extracted with ether, and the ether extract was washed (H_2O) and dried (Na_2SO_4). Removal of the ether followed by distillation of the residual oil gave the acetylacetonitriles as colorless viscous liquids which crystallized slowly on standing. Infrared spectra of these compounds are listed in Table III.

TABLE III

INFRARED ABSORPTION SPECTRA OF β-KETONITRILES



	Absorption, cm^{-1}		
R	Hydrogen bonding	C≡N	C=O
2-CH_3	3100–3650	2225	1760
2-Cl	3050–3700	2220	1758
2-F	3050-3700	2220	1760
6-CH_3	3100–3700	2210	1750

Substituted 3,4-Dimethoxyphenylacetones.—The viscous β-ketonitriles were warmed slightly to make them mobile before mixing them with H_2SO_4. To a cold solution of 39 ml of H_2SO_4 and 10 ml of water was added wih stirring 0.075 mole of the ketonitrile. The reaction mixture was warmed with stirring over a 10-min period to the temperature indicated below for each case, and maintained at that temperature as directed:[18] for the 2-methyl derivative, 90°, 10 min; 2-chloro, 100°, 10–15 min; 6-methyl, 100°, 20 min; 2-fluoro, 90°, 20 min. The reaction mixture was cooled and diluted with 120 ml of water and warmed at 95–100° with stirring for about 3 hr. It was then cooled, diluted with water, and extracted with ether. After washing (dilute $NaHCO_3$, H_2O) and drying (Na_2SO_4), the ether was distilled. The oily ketones were distilled and further characterized as their 2,4-dinitrophenylhydrazones.

The acid hydrolysis of the 2-fluoro derivative gave an oil whose infrared spectrum contained an OH band. However, all reactions of this material furnished products in excellent yields, identical with those formed from 2-fluoro-3,4-dimethoxyphenylacetone. The molecular weight of the ketone is 212, and that of the substance with the OH band was found to be 213 (osmometric method). The compound is therefore the enol of the ketone, stabilized by the *o*-fluorine atom.

4-Methyl-4-(2- or 6-substituted 3,4-dimethoxybenzyl)hydantoins.—A mixture of 0.05 mole of the ketone, 0.45 mole of $(NH_4)_2CO_3$, 0.07 mole of KCN, and 325 ml of 50% aqueous ethanol was stirred and heated at 58–62° for 8 hr. After stirring for another 12 hr at room temperature the reaction mixture was concentrated *in vacuo* to half its volume and chilled in an ice bath. The separated hydantoin was filtered off, washed with cold water, and recrystallized.

Substituted α-Methyl-3,4-dimethoxyphenylalanines.—A solution of 0.01 mole of the hydantoin in 425 ml of water and 0.05 mole of $Ba(OH)_2 \cdot 8H_2O$ was refluxed for about 65 hr, cooled, and adjusted with stirring to pH 1.5 with 2 *N* H_2SO_4. The solution was filtered, the $BaSO_4$ was washed thoroughly with hot water, and the combined filtrates were concentrated to a syrup under reduced pressure. After addition of ethanol and reconcentration the syrupy residue was dissolved in acetone, adjusted to about pH 9 by dropwise addition of diethylamine, and again brought back to pH 6–6.5 with glacial acetic acid. The amino acid was filtered from the cooled solution, washed with acetone and ethanol, cleared with activated charcoal, and recrystallized from water. The analytical samples were dried over P_2O_5 at 110 or 140° (0.025 mm) for 36 hr.

Substituted α-Methyl-3,4-dihydroxyphenylalanines.—The dimethoxyamino acid (2.0 g) was refluxed in 20 ml of 48% HBr for 12–14 hr. After concentration under reduced pressure and addition of acetone the residue was reconcentrated and then dissolved in 30 ml of acetone. The solution was adjusted to pH 8.5 by dropwise addition of diethylamine with stirring and then brought back to pH 5.7–6.0 by careful addition of glacial acetic acid with stirring. The solution was left overnight at 27°; the crystalline amino acid was filtered off, washed with three portions of acetone, and dried. The crude material was dissolved in distilled water containing a little SO_2 and treated with charcoal at about 95°. After filtration, the solution was concentrated to a syrup at the aspirator.

Hydrochlorides were prepared by suspending 300 mg of the analytically pure amino acid in 3 ml of absolute ethanol and passing a small amount of dry HCl with swirling over the suspension. The salt thus formed went into solution and was precipitated by careful addition of dry ether. The colorless crystals of the salt were filtered off and washed with anhydrous ether.

Inhibition of Dopa Decarboxylase.—Hog kidney dry powder was freshly prepared by the method of Schales and Schales.[19] Release of $^{14}CO_2$ from DL-dopa-$^{14}CO_2H$ was estimated by the method of Aures and Clark.[20]

The incubation mixture consisted of 0.05 mg of kidney dry powder, 0.0102 μmole of pyridoxal 5-phosphate, 0.5 μmole of reduced glutathione, and 0.04 μcurie of DL-dopa-$^{14}CO_2H$ which was adjusted to a total of 1.0 μmole with unlabeled DL-dopa. The inhibitors were added in a 0.01 *N* HCl solution in different concentrations. Sodium phosphate buffer was added to adjust the pH to 7.0 and 0.06 *M*. The total volume was 0.5 ml. Various blanks were used: (a) reagent blank, the above minus the enzyme preparation; (b) inhibited blank, identical with the above except 0.5 μmole of semicarbazide was added; (c) boiled blank, identical with the above, except the enzyme preparation was heat inactivated for 10 min at 100°. Enzyme, pyridoxal phosphate, glutathione, buffer, and inhibitor were incubated for 10 min at 37° under N_2, then the dopa was added. After the addition of the substrate (dopa), the reaction was allowed to proceed for 30 min and then stopped by freezing.

Under the experimental conditions employed, there was a linear relationship between $^{14}CO_2$ release and the amount of enzyme preparation, up to 0.1 mg of dry powder.

Tyrosine Hydroxylase Inhibition Tests.—Rat brain (minus cerebellum) (1.2 g) was homogenized in 4.8 ml of 0.25 *M* sucrose

(16) L. Friedman and H. Shechter, *J. Org. Chem.*, **25**, 877 (1960).

(17) For experiments conducted in methanol, *cf.* R. H. F. Manske and A. E. Ledingham, *Can. J. Res.*, **17B**, 14 (1939).

(18) Hydrolysis at 80° did not remove the cyano group completely.

(19) O. Schales and S. S. Schales, *Arch. Biochem.*, **24**, 83 (1949).

(20) D. Aures and W. G. Clark, *Anal. Biochem.*, **9**, 35 (1964).

solution. A mixture of 0.1 ml of this homogenate, 0.1 ml of 0.2 *M* phosphate buffer, pH 6.4 (containing the test compound where indicated), and 0.1 ml of a mixture of 1.5 ml of labeled tyrosine, 0.6 ml of water, and 0.9 ml of tyrosine (26.6 μg/ml) was incubated. Final compound concentrations were 10^{-4} *M* at which concentration DL-phenylalanine, DL-*p*-fluorophenylalanine, and DL-α-methyl-*m*-tyrosine decreased the conversion to about 25% of the control. Only **32** showed some activity (24% decrease of conversion) but less than α-methyldopa.

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Chemistry of Cephalosporin Antibiotics. V.[1] Amides and Esters of Cephalothin[2]

ROBERT R. CHAUVETTE AND EDWIN H. FLYNN

The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana

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Chemical alteration of cephalothin (I) which might lead to orally active derivatives was investigated by preparing a number of C-4 carboxyl modifications.[3] Isomerization of the double bond in the thiazine ring was encountered under many conditions of amidation and esterification, giving rise to Δ^2-cephalosporins which were completely devoid of antimicrobial activity.

7-(Thiophene-2-acetamido)cephalosporanic acid (I) is an outstanding member of a series of cephalosporins prepared some time ago in these laboratories.[4] There are many reports of its desirable antibacterial activities against gram-positive and gram-negative organisms and penicillin-resistant staphylococci in the laboratory and in clinical practice.[5] As cephalothin and many of its analogs lack oral efficacy, an investigation of the effect of modification at the C-4 carboxyl group on oral absorption and biological activity was undertaken.

Both amides and esters were considered. An interesting speculation was the possibility that amides derived from a cephalosporanic acid and an amino acid might cross the intestinal wall and be cleaved in the body. Although simple esters, like the methyl ester, are known to possess diminished antibiotic activity compared to the free acids,[4] the possibility exists that more easily hydrolyzable esters (by enzymatic or chemical means) might exhibit significant *in vivo* activity. A therapeutic advantage might be anticipated from derived compounds if the structural environment of the carboxyl group is a bar to absorption through the gastric or intestinal walls. Activity could be inherent in the derivative or be produced as a result of enzymatic cleavage to the parent compound after absorption has occurred. Gastric acidity, often a negative influence in oral absorbability of penicillins, would seem to be an unlikely factor in cephalosporin absorption because of the relatively good acid stability of this class of antibiotics. Objectives similar to these are not uncommon in the literature of penicillin chemistry.[6] A second motivation for this work was provided by a recurring need for an easily cleaved blocking group for the carboxylic acid in cephalosporin synthetic chemistry.

This paper reports the chemistry involved in amidations and esterifications of 7-(thiophene-2-acetamido)-cephalosporanic acid (I).

To form peptides from a cephalosporin required that the carboxyl at C-4 be appropriately activated for acylation of a protected amino acid. Nefkens, *et al.*,[7] have demonstrated that N-hydroxyphthalimide condenses with carboxylic acids, in the presence of a carbodiimide, to give oxyphthalimide esters that are suitable intermediates in peptide synthesis. Using their conditions, I was treated with N-hydroxyphthalimide to yield the expected cephalosporanoyloxyphthalimide (III) in respectable yield. The isolable products from reaction of III with a number of amines, however, were not the anticipated Δ^3-cephalosporinamides. With ethyl glycinate, for example, III gave a good yield of IV in which the thiazine ring double bond had completely isomerized to the Δ^2 position.

Ready isomerization to Δ^2-cephalosporins accompanied many of the reactions included in this study. Identification of these Δ^2 isomers was possible from the characteristics which follow. (1) The ultraviolet absorption near 260 mμ, which is correlated with β-lactam double bond conjugation in the normal Δ^3-cephalosporin ring system, is lacking. (2) In the nmr spectra, lone protons at C-2 and C-4, visible as single peaks near τ 3.6 and 5.0, respectively, replace the methylene protons adjacent to the sulfur, evident as doublets centered near τ 6.4 and 6.7 in the normal Δ^3-cephalosporin series. Further, the centers of the single-proton quartet and the single-proton doublet

(1) Paper IV: E. Van Heyningen and C. N. Brown, *J. Med. Chem.*, **8**, 174 (1965).

(2) Cephalothin is the generic name for 7-(thiophene-2-acetamido)cephalosporanic acid; cephalothin sodium salt, Keflin®.

(3) For naming and numbering of the cephalosporins, see R. B. Morin, B. G. Jackson, E. H. Flynn, and R. W. Roeske, *J. Am. Chem. Soc.*, **84**, 3400 (1962).

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(7) G. H. L. Nefkens, G. I. Tesser, and R. J. F. Nivard, *Rec. Trav. Chim.*, **81**, 683 (1962).