Cyclic Methyldopa Analogs as Potential Antihypertensive and Antineoplastic Agents

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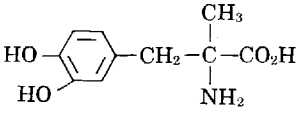
Three cyclic methyldopa analogs (III*a*, *b*, and *c*) were synthesized by hydrolysis of the appropriate spirohydantoins (V), which were obtained through the Bucherer synthesis. Amino acids (III*a* and *b*) and hydantoins (V*a* and *c*) were inactive against experimental tumors. The 5 hydantoins tested were devoid of anticonvulsant effect.

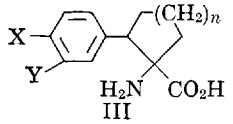
THE AMINO acid, methyldopa (I), is a recognized drug for hypertension (1), while another amino acid, 1-aminocyclopentane-1-carboxylic acid (NSC-1026, II), possesses inhibitory activity against



I

II

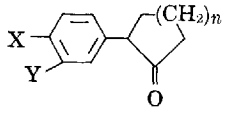
tumors (2). The structures of these substances, which are aliphatic α-amino acids fully substituted at the α position, suggested the synthesis of hybrids of I and II which are represented by structure III.



III

a, ($n = 1$, X = Y = H)
b, ($n = 2$, X = Y = H)
c, ($n = 2$, X = Y = OH)

The approach to compounds of type III was through appropriate ketones IV which were converted by



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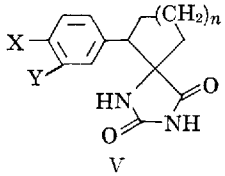
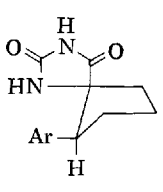


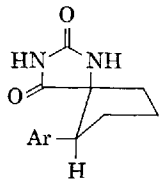
V

means of potassium cyanide and ammonium carbonate in a Bucherer-Bergs synthesis (3) to the spirohydantoins (V*a–e*) of Table I. Demethylation of hydantoins V*b*, *d*, and *e* to phenolic spirohydantoins (V*f*, *g*, and *h*) of Table I was effected by means of refluxing overnight with 47% hydriodic acid and glacial acetic acid. All the hydantoins of Table I exhibited characteristic infrared absorption at 3.1 μ and a doublet in the carbonyl region between 5.6 and 5.9 μ.

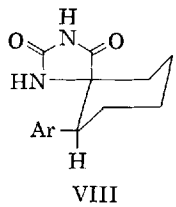
Hydantoins of structure V are representable by the following structures as *cis-trans* pairs (VI and VII; VIII and IX). However, separation of any



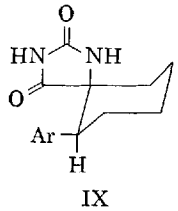
VI



VII



VIII



IX

isomers was not possible by means of thin-layer chromatography. In each case only a single spot was observable. This observation agrees with the recent conclusion (4) that the Bucherer-Bergs synthesis is stereoselective and only a small percentage yield of a second isomer is sometimes isolable.

Methods employing barium hydroxide or hydrogen chloride in glacial acetic acid failed to hydrolyze the spirohydantoins (V). Despite poor solubility, hydantoins (V*a* and V*c*) were hydrolyzed by heating at 140–150° with 60% sulfuric acid (5). After neutralization of the mixture with barium carbonate and desalting by ion-exchange resin, the yield of amino acid was 33% of III*a* and 51% of III*b*. The *N*-(2,4-dinitrophenyl) derivative of each amino acid was prepared. Amino acid (III*c*) was obtained by hydrolysis of hydantoin (V*e*) and demethylation of the unisolated intermediate by means of hydriodic acid. The crude hydriodide was converted to the hydrochloride of III*c* by ion exchange. Over-all yield from the hydantoin was 12%.

PHARMACOLOGICAL RESULTS

The following summary of results available to date was prepared from reports submitted by Dr. Joseph Leiter, Cancer Chemotherapy National Service Center, Bethesda, Md. Detailed information concerning test procedures may be found in publications from that office (6).

Amino acids (III*a* and *b*) and spirohydantoins V*a* and *c* were screened by the Southern Research Institute, Birmingham, Ala., and were all found to be nontoxic and inactive against sarcoma 180, solid Friend virus leukemia, and lymphoid leukemia L-1210 in mice and also inactive in cell culture.

Professor Ewart Swinyard, College of Pharmacy, University of Utah, found that spirohydantoins V*a*, *c*, *f*, *g*, and *h* were devoid of appreciable anti-

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TABLE I.—6-ARYL-1,3-DIAZASPIRO[4.4]NONANE-2,4-DIONES (V, n = 1) AND 6-ARYL-1,3-DIAZASPIRO[4.5] DECANE-2,4-DIONES (V, n = 2)

Compd.	Aryl	n	M.p., °C.	% Yield	Molecular Formula	Anal., % Calcd.	Found
V*a*	Phenyl	1	215–217 dec.	61	$C_{13}H_{14}N_2O_2$	C, 67.81	67.72
						H, 6.13	6.16
						N, 12.17	12.28
V*b*	4-Methoxyphenyl	1	226–229 dec.	46	$C_{14}H_{16}N_2O_3$	C, 64.60	64.66
						H, 6.20	6.26
						N, 10.76	10.79
V*c*	Phenyl	2	250–252 dec.	60	$C_{14}H_{16}N_2O_3$[a]	C, 68.61	68.61
						H, 6.61	6.46
						N, 11.47	11.30
V*d*	4-Methoxyphenyl	2	235–240 dec.	65	$C_{15}H_{18}N_2O_3$	C, 65.67	65.66
						H, 6.61	6.45
						N, 10.21	10.10
V*e*	3,4-Dimethoxyphenyl	2	221–224 dec.	58	$C_{16}H_{20}N_2O_4$	C, 63.14	62.92
						H, 6.62	6.57
						N, 9.21	9.35
V*f*	4-Hydroxyphenyl	1	345–350 dec.	62	$C_{13}H_{14}N_2O_3$	C, 63.40	63.14
						H, 5.73	5.94
						N, 11.38	11.56
V*g*	4-Hydroxyphenyl	2	389–392 dec.	67	$C_{14}H_{16}N_2O_3$	C, 64.60	64.64
						H, 6.20	6.27
						N, 10.76	10.74
V*h*	3,4-Dihydroxyphenyl	2	317–328 dec.	64	$C_{14}H_{16}N_2O_3$	C, 60.86	60.99
						H, 5.84	5.84
						N, 10.14	10.02

[a] Product analyzed correctly for monohydrate until dried at 150° (0.3 mm.). Previously reported by Tiffeneau, M., Tchoubar, B., Saiaslambert, M., and LeTellier-Dupré, M., *Bull. Soc. Chim. France*, **1957**, 445; m.p. 255°, yield 25%.

convulsant activity in mice as measured by maximal electroshock seizure test.

Owing to the difficulty of obtaining screening of compounds obtained under a grant from the National Institutes of Health, no data are available pertaining to possible antihypertensive activity of the amino acid (III).

EXPERIMENTAL

2 - Arylcycloalkanones.—2 - Phenylcyclopentanone (7), 2-phenylcyclohexanone (8), and 2-(4-methoxyphenyl)-cyclohexanone (9) were obtained as described in the literature. 2-(4-Methoxyphenyl)-cyclopentanone had not been prepared before. The method described in *Reference 9* was used, but difficulty was encountered in its isolation. Its identity was established by formation of the hydantoin (V*b*). 2-(3,4-Dimethoxyphenyl)-cyclohexanone, a new compound, was obtained from 3,4-dimethoxybenzaldehyde by a general method (10), but similarly the hydantoin (V*e*) was made from the crude ketone.

Spirohydantoins (Table I).—The general procedure of preparation was based upon that of Henze and Speer (11). The appropriate ketone was mixed with 2 equivalents of potassium cyanide and 4 equivalents of ammonium carbonate in about 300 ml. of 50% alcohol per 0.1 mole of ketone. In the case of V*c*, the starting ketone was first dissolved in 50 ml. of alcohol. The mixture was stirred for about 4 to 5 hr. at 58–60°. Cooling the mixture to room temperature yields a pure first crop. Furthermore, less pure product was obtained by evaporation of the filtrate to about two-thirds of its *volume and* then acidifying. (Hood!) The hydantoins were recrystallized from 50% alcohol.

Thin-layer chromatography of hydantions, using silica gel as adsorbent and with a developing time of 50 min., gave the following R_f values of approximately the same magnitude, for solvent system chloroform/acetone (1:1) and chloroform/acetone (2:1), respectively: V*a*, 0.83 and 0.59; V*c*, 0.79 and 0.54; and V*e*, 0.77 and 0.49. Sulfuric acid spray and heat were used for development of spots.

Demethylation of Hydantoins Vb, d, and e.—A mixture of 0.01 mole of the methoxyphenylspirohydantoin, 20 ml. of 47% hydriodic acid, and 20 ml. of glacial acetic acid was heated at reflux overnight. After the mixture was cooled and diluted with water, crystallization was induced. Evaporation of the mother liquors gave less pure product.

1 - Amino - 2 - phenylcyclopentane - 1 - carboxylic Acid (IIIa).—A mixture of 23 Gm. (0.1 mole) of crude hydantoin (V*a*) and 400 ml. of 60% sulfuric acid was heated with stirring for 6 hr. at 140–150°. The cooled solution was neutralized with barium carbonate, the precipitated barium sulfate was removed by filtration and washed several times with hot water. From the hot water 2.3 Gm. of unchanged V*a* separated. The solution was concentrated and desalted by absorption of the amino acid in a strongly acidic ion-exchange resin (Dowex 50), washing out the anions and replacing the amino acid with 2 *N* ammonia. When, upon concentrating, the amino acid began to crystallize, an equal volume of hot alcohol was added. Cooling gave 6.9 Gm. (34% yield) of III*a*, m.p. 290° dec. With paper chromatography, only 1 spot was obtained. Whatman's No. 1 paper was employed in ascending flow of solvent at room temperature for the following R_f values: 0.86 (120 butanol/30 glacial acetic acid/50 water); 0.89 (160 phenol/40 water); 0.83 (150 phenol/40 ethanol/10 water); 0.70 (60 butanol/60 pyridine/60 water). Ninhydrin spray was used for development.

Anal.—Calcd. for $C_{12}H_{15}NO_2$: C, 70.22; H, 7.37; N, 6.82. Found: C, 70.18; H, 7.41; N, 6.85.

N - (2,4 - Dinitrophenyl) - 1 - amino - 2 - phenylcyclopentane - 1 - carboxylic Acid.—This was made from III*a* and 2,4-dinitrophenyl-1-fluorobenzene (12). Two recrystallizations from dilute alcohol gave a product, m.p. 220–230° dec. Paper chromatography produced tailing. Thin-layer chromatography using Silica Gel G as adsorbent and solvent system 95 chloroform/5 methanol/1 glacial acetic acid gave after 50 min. R_f value 0.70 (single spot).

Anal.—Calcd. for $C_{18}H_{17}N_3O_6$: C, 58.45; H, 4.09; N, 11.38. Found: C, 58.68; H, 4.39; N, 11.20.

1 - Amino - 2 - phenylcyclohexane - 1 - carboxylic Acid (IIIb).—As in the preparation of III*a*, 26.2 Gm. (0.1 mole) of crude hydantoin V*c* was hydrolyzed. After 6 hr., 38% of V*c* was recovered, 28% after 24 hr., and none remained after 2 days of heating but carbonization resulted. In the last case, the yield of III*b* was 51%, m.p. 286–290° dec. Paper chromatography gave only 1 spot, and R_f values for the same solvent system used for III*a* were, respectively, 0.87, 0.85, 0.82, and 0.73.

Anal.—Calcd. for $C_{13}H_{17}NO_2$: C, 71.20; H, 7.82; N, 6.39. Found: C, 71.02; H, 8.02; N, 6.36.

N - (2,4 - Dinitrophenyl) - 1 - amino - 2 - phenylcyclohexane-1-carboxylic Acid.—This was made (12) as a derivative of III*b*, m.p. 204–206° dec., after 2 crystallizations from 50% alcohol. R_f 0.68 (thin-layer, adsorbent Silica Gel G, 95 chloroform/5 methanol/1 glacial acetic acid).

Anal.—Calcd. for $C_{19}H_{19}N_3O_6$: C, 59.22; H, 4.97; N, 11.16. Found: C, 59.34; H, 5.05; N, 11.10.

1 - Amino - 2 - (3,4 - dihydroxyphenyl) - cyclohexane-1-carboxylic Acid (IIIc) Hydrochloride.—A mixture of 20 Gm. (0.062 mole) of crude hydantoin (V*e*) and 400 ml. of 60% sulfuric acid was stirred and heated at 135–140° for 2 days. The dark solution was neutralized with barium carbonate. The precipitated barium sulfate was removed by filtration, washed with hot water, and the combined solutions evaporated to dryness to give 10 Gm. of dark brown material. It was dissolved in 100 ml. of 47% HI and heated at reflux for 24 hr. Diluted with water and filtered, the brown solution was extracted with ether. The aqueous layer was evaporated to dryness. The product was dried under reduced pressure to remove excess hydrogen iodide, after which it was dissolved in water and adsorbed on an ion-exchange column (Dowex 50). The iodide ions were removed with water and the amino acid eluted with 4 *N* hydrochloric acid. The solution was evaporated to dryness to give 3 Gm. of a slightly gray solid. It was purified by several reprecipitations from glacial acetic acid by ether. A yield of 1.8 Gm. (10% from V*e*) of slightly tan solid III*c* hydrochloride was obtained, m.p. > 300°.

The product is acidic and gives a dark green color with ferric chloride. I.R. spectrum shows a broad band at 2.8 to 4.55 μ, suggesting phenolic and carboxylic hydroxyls, C—H and RNH_3^+ absorption. A carbonyl peak appears at 5.8 μ.

Anal.—Calcd. for $C_{13}H_{18}ClNO_4$: C, 54.20; H, 6.31; N, 4.87. Found: C, 54.34; H, 7.00; N, 4.65.

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