against the composition of the mobile phase, which showed a linear relationship. According to Boyce and Milborrow,[19] the Rm values in the range of linearity were considered to be the most satisfactory ones. By means of the least-squares method, the equations of the straight lines were calculated. On the other hand, the equation of G-I (17) from 20 experiments had been derived as $Rm_c = -0.0172c + 0.7018$ (c = concentration; $r = 0.998$), from which the Rm_{50} of 17 could be calculated as −0.16. This was defined as a standard Rm_{50} value. Whenever TLC of other compounds were developed, G-I (17) was spotted simultaneously as a standard. And when Rm_{50} values of other grayanotoxins were calculated from their equations, the concentration corresponding to the standard Rm_{50} value (−0.16) was used instead of 50 in order to avoid any error from experimental conditions. Rm_{50} values thus obtained were listed in Table II.

Acknowledgment. This work was supported in part by a Grant-in-Aid for Scientific Research by The Ministry of Education, Science and Culture of Japan. The authors are very grateful to K. Tagami for assistance in the experimental work.

Registry No. 1, 4678-45-9; **5a**, 23984-17-0; **5b**, 84849-09-2; **5c**, 84849-10-5; **5d**, 84849-11-6; **5e**, 84849-12-7; **5f**, 84849-13-8; **5g**, 84849-14-9; **5h**, 84849-15-0; **5i**, 84849-16-1; 11, 54781-72-5; **13a**, 84849-17-2; **13b**, 84849-18-3; **13c**, 84849-19-4; **13d**, 84849-20-7; **13e**, 84849-21-8; **13f**, 84849-22-9; **13g**, 84849-23-0; **13h**, 84849-24-1; **13i**, 84849-25-2; 14, 54781-61-2; 15, 84863-60-5; 16, 84849-26-3; 17, 4720-09-6; 18, 84893-93-6; 2,3,4,6-tetraacetyl-β-D-glucopyranosyl bromide, 6919-96-6; 3,6-dibenzoyl-14-*O*-(tetraacetyl-β-D-glucopyranosyl)grayanotoxin III, 84863-61-6.

(19) C. B. C. Boyce and B. V. Milborrow, *Nature (London)*, **208**, 537 (1965).

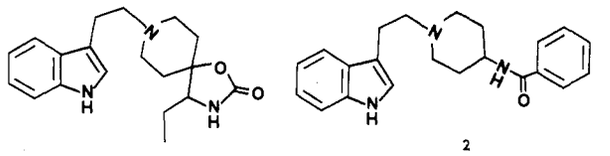
Antihypertensive 9-Substituted 1-Oxa-4,9-diazaspiro[5.5]undecan-3-ones[1]

Robin D. Clark,*[†] Joan M. Caroon,[†] David B. Repke,[†] Arthur M. Strosberg,*[‡] Susan M. Bitter,[‡] Marlys D. Okada,[‡] Anton D. Michel,[§] and Roger L. Whiting[§]

Institute of Organic Chemistry and Institute of Pharmacology and Metabolism, Syntex Research, Palo Alto, California 94304, and Syntex Research Centre, Edinburgh, Scotland. *Received August 16, 1982*

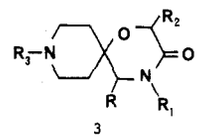
Forty-one 9-substituted 1-oxa-4,9-diazaspiro[5.5]undecan-3-ones were prepared for antihypertensive screening in the spontaneously hypertensive rat (SHR). For the 9-(2-indol-3-ylethyl) series, the parent compound, 9-(2-indol-3-ylethyl)-1-oxa-4,9-diazaspiro[5.5]undecan-3-one (21), was the most potent antihypertensive agent. Substitution of lower alkyl groups on the spirolactam ring gave compounds close in activity to 21, while substitution with large alkyl or aryl groups led to a significant decrease in activity. Ring-opened analogues of 21 that contained the same functionality were markedly less active. Several 1-oxa-4,9-diazaspiro[5.5]undecan-3-ones substituted at the 9 position with 1,4-benzodioxan-2-ylmethyl, 1,4-benzodioxan-2-ylhydroxyethyl, and 2-phenylethyl groups also demonstrated significant activity. Compound 21 was chosen for a detailed pharmacological evaluation. Its antihypertensive activity appears to be predominantly due to peripheral α_1-adrenoceptor blockade.

The synthesis and antihypertensive activity of a series of spiropiperidinyloxazolidones exemplified by 1 have been



1

2

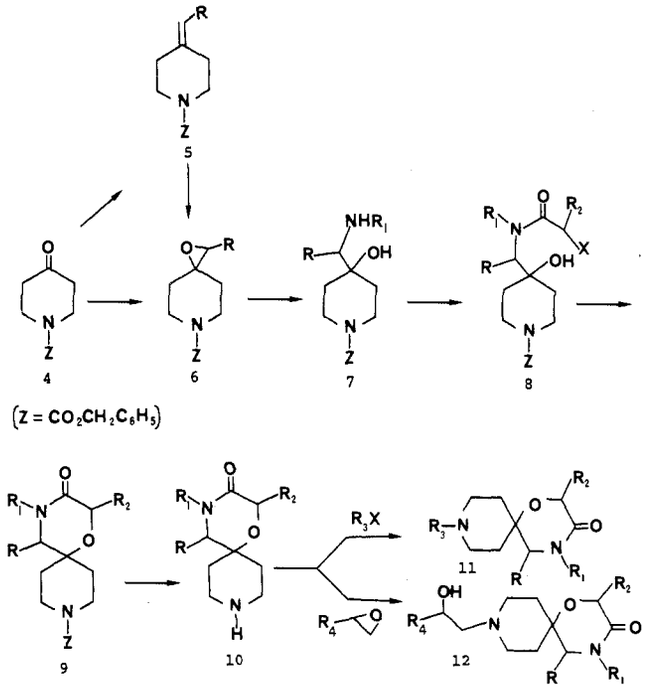


3

recently described.[2] The antihypertensive activity of this series was due to postsynaptic (α_1) adrenoceptor blockade as has been found for the structurally related antihypertensive agent indoramin (2).[3] It was thus of interest to examine the related 9-substituted 1-oxa-4,9-diazaspiro-[5.5]undecan-3-ones of general formula 3,[4] and we now report that a number of compounds from this series display significantly greater antihypertensive activity relative to the earlier series. With the activity of the spiro compounds established, it was of further interest to prepare acyclic analogues that contain the same basic functionality in order to establish the exact structural requirements for activity.

Chemistry. The 1-oxa-4,9-diazaspiro[5.5]undecan-3-one ring system was prepared as shown in Scheme I. Epoxides

Scheme I



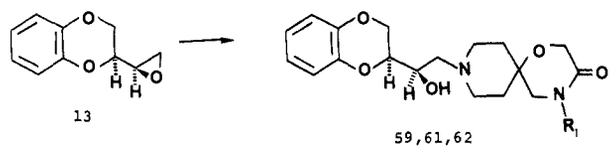
6 were prepared from 1-carbobenzoxypiperidone 4[2] either directly with a sulfur ylide for compounds in which R =

[†] Institute of Organic Chemistry.
[‡] Institute of Pharmacology and Metabolism.
[§] Syntex Research Centre.

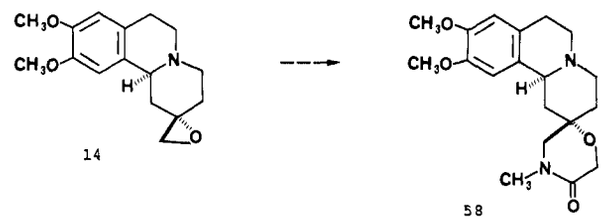
(1) Contribution No. 625 from the Institute of Organic Chemistry.
(2) Caroon, J. M.; Clark, R. D.; Kluge, A. F.; Nelson, J. T.; Strosberg, A. M.; Unger, S. H.; Michel, A. D.; Whiting, R. L. *J. Med. Chem.* 1981, *24*, 1320.

0022-2623/83/1826-0855$01.50/0

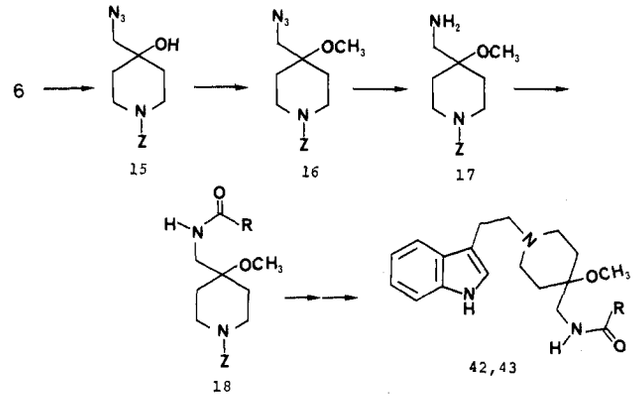
Scheme II



Scheme III



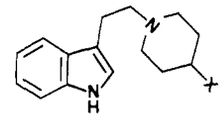
Scheme IV



H or via conversion to the olefin, followed by epoxidation with *m*-chloroperoxybenzoic acid for the series in which R = alkyl. Use of dimethylsulfoxonium methylide[5] gave significantly better yields than did dimethylsulfonium methylide. Heating the epoxides **6** with an amine, either neat or in a metal bomb, gave the amino alcohols **7**, which were acylated with α-halo carboxylic acid chlorides to afford amides **8**. Ring closure to the spirocycles **9** was accomplished straightforwardly in the cases in which R = alkyl by utilizing sodium hydride in dimethoxyethane. However, in the case of R_1 = H, significant formation of the dimeric diketopiperazine was observed under these conditions. This problem was avoided by addition of the α-chloro amide **8** to a warm solution of 2 equiv of potassium *tert*-butoxide in *tert*-butyl alcohol or THF, which resulted in quantitative conversion to **9**. Catalytic hydrogenolysis or treatment with HBr–acetic acid gave the spiropiperidines **10**. Reaction of **10** with the requisite alkyl halide gave compounds **11**, while compounds **12** were obtained from the corresponding epoxide. Compound **57** was prepared by reductive amination of β-tetralone. The *erythro*-benzodioxanyl epoxide **13** (Scheme II) was prepared according to the literature procedure from catechol and the bis(chloromethyl)oxirane.[6] The approximately 1:1 erythro–threo mixture **60** was prepared from the bromocarbinol as previously described.[2] The benzoquinolizine **58** was prepared by utilizing the same sequence of steps from the epoxide **14**[7] (Scheme III).

For the preparation of **42** and **43** (Scheme IV), epoxide **6** was opened with sodium azide, and the azido alcohol thus obtained was converted to the methyl ether **16** with methyl iodide–sodium hydride. Controlled hydrogenation of **16** gave the amino alcohol **17**, which was acylated, deprotected, and alkylated with 2-indolylethyl bromide.

Compounds **38–41** were prepared by acylation of amine



19, X = $CONH_2$
20, X = CH_2NH_2
38–41, X = CH_2NHCOR

20, which was obtained by lithium aluminum hydride reduction of the amide **19** derived from reaction of 2-indolylethyl bromide with isonipecotamide.

Results and Structure–Activity Relationships

The compounds in Table I were tested for antihypertensive activity in male, Okamoto-Aoki strain, spontaneously hypertensive rats (SHR). The data in Table I represent the percentage decrease in systolic blood pressure for the drug-treated group relative to the value for the untreated control.

In the indolylethyl series, the most active compound was the unsubstituted parent compound **21**. Since we,[8] and others,[3,9] have previously shown in related indolyl series that substitution in the indole moiety, variation of the length of the alkyl chain to one or three carbons, or alkylation in the piperidine ring led to substantial diminution of activity, such modifications were not performed in the present series. The only substitution performed was, therefore, in the lactam ring. Substitution at either position 5 (R) or 2 (R_2) with alkyl or aryl groups decreased activity, and the decrease was directly related to the size of the substituent. The 5-substituted compounds were significantly more active, and the methyl analogue **22** was close to the parent **21** in potency. Substitution at the nitrogen (position 4) with lower alkyl led to compounds **25–27**, which, while still retaining substantial activity, were somewhat less active than **21**. Again, placement of larger groups, as in **28** gave substantial drop-off in activity. The *N*-phenyl amide **29** was highly active at the high dose (50 mg/kg), but this activity dropped off sharply at lower doses. Multiple alkylation at positions 2 and 4 or positions 4 and 5 led to virtually inactive compounds.

To ascertain if the spirolactam was necessary for maximal activity, we evaluated the acyclic congeners **40** and **42**, which retain the same functionality but differ in their connectivity. Neither approached the spirolactam **21** in activity. In both cases, analogues in which methyl was replaced by phenyl (**41** and **43**) proved to be more active, in contrast to the trend in the spiro series. Thus, incorporation of the amide group into a spirolactam is clearly necessary for maximal activity. This could be due to receptor binding, which requires the rigid and compact spiro ring for optimal fit. Another intriguing (and highly spectulative) possibility is that the acyclic amides are metabolized by amide hydrolysis, which, for example, in the case of **38–41** would yield the moderately active amine

(3) Archibald, J. L.; Alps, B. J.; Cavalla, J. F.; Jackson, J. L. *J. Med. Chem.* **1971**, *14*, 1054.
(4) One example of a compound with this ring system has been reported: Maillard, J.; Langlois, M.; Van Tri, V.; Delaunay, P.; de Chenu, J.; Roussillon, J. L.; Morin, R.; Benharkate, M.; Manuel, C.; Motosso, F. *Chim. Ther.* **1973**, 393.
(5) Corey, E. J.; Chaykovsky, M. *J. Am. Chem. Soc.* **1965**, *87*, 1353.
(6) Gschwend, H. W.; Huebner, C. F. U.S. Patent 4 212 808, 1980.
(7) Davis, R.; Kluge, A. F.; Maddox, M. L.; Sparacino, M. A. *J. Org. Chem.* **1983**, *48*, 255.
(8) Reference 2 and unpublished results.
(9) Glamkowski, E. J.; Reitano, P. A.; Woodward, D. L. *J. Med. Chem.* **1977**, *20*, 1485.

20. Hydrolysis of the spirolactam would yield an amino acid that could presumably reclose, or be in equilibrium with, the active parent spirolactam.[10]

Substituting at position 9 of the diazaspiro[5.5]undecane with groups other than indolylethyl gave several other active series. The phenylethyl analogue **44** was highly active at 12.5 mg/kg but inactive at 6.25 mg/kg. Substituents at either the 3 or 4 position of the phenyl ring led to inactive compounds. The 9-(4-fluorobutyrophenones) **54–56** were also active, with maximal activity associated with the *N*-methyl analogue **55**. However, there was some toxicity associated with this compound at 50 mg/kg. Interestingly, the indolylethyl analogue **25** also showed some toxicity and was the only compound in that series to do so.

In the benzodioxanylhydroxyethyl series, the *N*-methyl compound **61** was significantly more active than the parent (**59**), unlike the trend in the indolylethyl series. The erythro isomer **61** was clearly more active than the erythro–threo mixture **60**. The benzodioxanylmethyl analogue **65** was significantly active for 3 h at 6.25 mg/kg and was considerably more potent that the homologue **66**.

On the basis of its activity in the SHR, **21** was chosen for more detailed evaluation. In the SHR, **21** lowered systolic blood pressure 20–30% for over 8 h at 6.25 mg/kg and 16–24% for over 2 h at 3.12 mg/kg. Its potency was therefore greater than the structurally related indoramin (**2**) but less than that of the potent α_1-blocker prazosin. The affinities of these three compounds for α adrenoceptors of rat cerebral cortex were determined by ligand binding using [^{3}H]prazosin[11] and [^{3}H]yohimbine[12] to label α_1 and α_2 adrenoceptors, respectively. The results are listed in Table II and are expressed as the negative log of the inhibition constant (K_I), which was calculated by the method of Cheng and Prusoff.[13] That all three compounds were antagonists at both α_1 and α_2 adrenoceptors was confirmed by using the rat, isolated, transversely bisected vas deferens as previously described.[2] From the values listed in Table II it is apparent that **21** is neither as potent nor as selective an α_1 blocker as prazosin or indoramin in this preparation. However, the extrapolation of in vitro α-blocking data to in vivo activity must be done with care.[14]

Further pharmacological evaluation of **21** has confirmed that its antihypertensive activity is due predominantly to peripheral α_1 blockade. Weak vasodilation unrelated to α_1 blockade has also been found to be induced by **21**. This compound has been chosen for development as an antihypertensive agent in man, and a complete description of its pharmacological evaluation will be presented in a forthcoming publication.

Experimental Section

Melting points (uncorrected) were obtained on a Fisher-Johns apparatus. Infrared spectra were recorded with a Perkin-Elmer 237B spectrometer; NMR spectra were obtained with Varian A-60 and Bruker WM 300 instruments. Mass spectra were obtained in either an Atlaswerke CH-4 or CH-7 instrument. Microanalyses were performed by Syntex Analytical Research and Atlantic Microlab, Atlanta, GA.

9-(2-Indol-3-ylethyl)-1-oxa-4,9-diazaspiro[5.5]undecan-3-one Hydrochloride (21). Epoxide **6** (R = H; 79 g, 320 mmol) in 1 L of 20% NH_3 in MeOH was heated in a metal bomb in a steam bath for 20 h. The bomb was cooled to room temperature, and the solution was removed and evaporated to afford 91.0 g (100%) of amino alcohol **7** as a colorless oil. This material was dissolved in 700 mL of ethyl acetate, and a solution of 100 g of K_2CO_3 in 750 mL of water was added. The mixture was stirred in an ice bath while 35 mL (440 mmol) of chloroacetyl chloride was added slowly, and stirring was continued for 30 min at room temperature. The ethyl acetate layer was separated and evaporated, and the residue was dissolved in 800 mL of acetone containing 100 g of NaI. The mixture was heated to reflux for 1 h, cooled, and filtered, and the filtrate was evaporated. The residue was filtered through silica gel with ethyl acetate to give 95 g (69% from **6**) of amide **8**, mp 109–110 °C.

A solution of 40 g (92.5 mmol) of **8** in 300 mL of THF was added slowly to a refluxing solution of 20 g (178 mmol) of potassium *tert*-butoxide in 600 mL of *tert*-butyl alcohol. The cooled solution was pooled with four similar reaction mixtures (144 g total of **8**) and neutralized with HOAc. Solvents were evaporated, and the residue was partitioned between water and ethyl acetate. The ethyl acetate was washed with brine, dried (Na_2SO_4), and evaporated to a solid residue. Trituration with ether afforded 90 g (93%) of lactam **9** (R, R_1, R_2 = H), mp 130–132 °C.

The lactam **9** (70 g, 240 mmol) was added to 400 mL of 1 M HBr in acetic acid. The mixture was stirred at room temperature for 1 h, and 200 mL of ethyl ether was added. The solid was filtered off and washed with ether to give 69 g (100%) of the HBr salt **10**, mp 205–207 °C dec.

A solution of 15 g (60 mmol) of **10** and 13.5 g (60 mmol) of 2-indolylethyl bromide in 75 mL of DMF and 20 mL of triethylamine was stirred at 65 °C for 20 h. The mixture was poured into dilute aqueous NH_4OH and extracted 3 times with CH_2Cl_2. The CH_2Cl_2 extracts were combined and washed twice with 5% aqueous HCl. The HCl extracts were made basic with NH_4OH and extracted with CH_2Cl_2. Evaporation of the CH_2Cl_2 and chromatography of the residue on silica gel (10% MeOH–CH_2Cl_2) gave 8.0 g (42%) of **21**: mp 48–50 °C; IR (KBr) 1675, 1350, 1100 cm^{-1}; NMR ($CDCl_3$) δ 1.60–2.00 (m, 4 H), 2.20–3.10 (m, 8 H), 3.23 (AB, J = 14 Hz, 2 H), 4.23 (s, 2 H), 7.00–7.83 (m, 6 H), 8.40 (br s, 1 H); MS, m/e 313 (M^+), 183, 130.

The HCl salt was prepared by dissolving the base in MeOH–HCl and inducing crystallization by addition of ether.

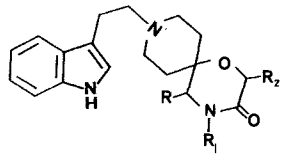
***erythro*-4-Phenyl-9-[2-(1,4-benzodioxan-2-yl)-2-hydroxyethyl]-1-oxa-4,9-diazaspiro[5.5]undecan-3-one Hydrochloride (62).** Epoxide **6** (R = H, 10 g, 40.5 mmol) and 18.6 g (200 mmol) of aniline were heated under nitrogen at 150 °C for 2 h. Excess aniline was removed by distillation with a Kugelrohr apparatus (40 mm), and the residue was chromatographed on silica gel with ether elution to give 13.5 g (88%) of **7** (R = H, R_1 = phenyl) as a thick oil. This material was dissolved in 200 mL of ethyl acetate, and a solution of 20 g of K_2CO_3 in 100 mL of water was added. The mixture was stirred in an ice bath while 5 mL of chloroacetyl chloride was slowly added. The precipitate was filtered, and the ethyl acetate was evaporated to a solid, which was combined with the original precipitate and triturated with ether to give 14 g (85%) of **8** (R = H, R_1 = phenyl, R_2 = H). This was dissolved in 200 mL of THF, and 4.2 g (37.5 mmol) of potassium *tert*-butoxide was added. The mixture was stirred for 1 h, and the solvent was then evaporated. The residue was partitioned between ethyl acetate and water. The ethyl acetate was dried (Na_2SO_4) and evaporated to a solid, which was hydrogenated in 200 mL of MeOH with 0.9 of 10% palladium on carbon at 60 psi for 1 h. The mixture was filtered, and the filtrate was evaporated to give 7.5 g (92.5% from **8**) of **10** (R = R_2 = H, R_1 = phenyl) as a thick oil.

A solution of 2.2 g (8.9 mmol) of **10** and 1.6 g (9 mmol) of *erythro*-epoxide 13[6] in 20 mL of MeOH and 40 mL of toluene was heated to reflux for 12 h. The solvents were evaporated, and the residue was chromatographed on silica gel (5% MeOH–CH_2Cl_2) to give 2.4 g (63%) of **62**: mp 50–51 °C; IR (KBr) 3650–3200, 1665, 1600, 1500, 1260 cm^{-1}; NMR ($CDCl_3$) δ 1.70 (m, 2 H), 2.05 (m, 2 H), 2.44 (td, J = 12 and 3 Hz, 1 H), 2.60 (dd, J = 12 and 9 Hz, 1 H), 2.63 (m, 1 H), 2.74 (dd, J = 12 and 3 Hz, 1 H), 2.76 (m, 1 H), 2.80 (dd, J = 12 and 3 Hz, 1 H), 3.59 (s, 2 H), 3.84 (m, 1 H),

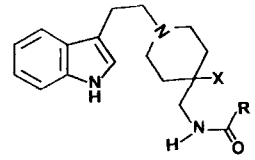
(10) The amino acid hydrolysis product of **21** was prepared (aqueous KOH, reflux) but had no oral activity in the SHR at 6.25 mg/kg: Becker, A.; Clark, R. D.; Strosberg, A. M., unpublished results.

(11) Greengrass, P.; Bremner, R. *Eur. J. Pharmacol.* **1979**, *55*, 323.
(12) Barnet, D. B.; Ferry, D. R.; Majid, M. H.; Nahorski, S. R.; Twigg, L. J. *Eur. J. Pharmacol.*, in press.
(13) Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.
(14) Massingham, R.; Dubocovich, M. L.; Shepperson, N. B.; Langer, S. Z. *J. Pharm. Exp. Ther.* **1981**, *217*, 467.

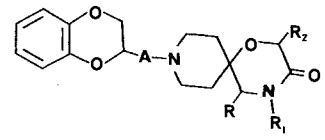
Table I. Antihypertensive Activity of 1-Oxa-4,9-diazaspiro[5.5]undecan-3-ones and Related Compounds



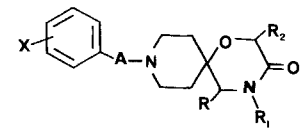
21–37



38–43



44–57



59–66

no.	R	R_1	R_2	yield,[a] %	mp,[b] °C	formula	anal.[c]	dose, mg/kg po	% fall in SBP[d] 1 h	2 h	3 h	4 h
21	H	H	H	42	270–273	$C_{18}H_{24}ClN_3O_2 \cdot 0.5H_2O$	C, H, N	50	53	48	48	41
								12.5	39	41	29	26
								6.25	28	30	20	19[f]
22	CH_3	H	H	40	175–177	$C_{19}H_{25}N_3O_2 \cdot 0.25H_2O$[e]	C, H, N	50	56	48	41	50
								12.5	24	36	24	*
23	CH_2CH_3	H	H	10	240–243	$C_{20}H_{28}ClN_3O_2 \cdot 0.25H_2O$	C, H, N	50	40	54	41	43
								12.5	21	25	*	*
24	C_6H_5	H	H	30	193–194	$C_{24}H_{28}ClN_3O_2 \cdot H_2O$	C, H, N	50	21	19	*	*
25	H	CH_3	H	44	153–155	$C_{19}H_{26}ClN_3O_2$	H, N; C[g]	50	50	44	34	26
								12.5	25	19	20	17
26	H	CH_2CH_3	H	13	280–286	$C_{20}H_{27}ClN_3O_2 \cdot 0.25H_2O$	C, H, N[h]	50	55	33	28	42
								12.5	22	33	30	21
27	H	$CH_2CH_2CH_3$	H	19	224–225	$C_{21}H_{30}ClN_3O_2 \cdot 0.25H_2O$	C, H, N	50	47	22	22	39
								12.5	18	26	24	16
28	H	$CH_2C_6H_5$	H	24	248–249	$C_{25}H_{30}ClN_3O_2 \cdot 0.25H_2O$	C, H, N	50	18	*	*	*
29	H	C_6H_5	H	59	245–247	$C_{24}H_{28}ClN_3O_2 \cdot 0.75H_2O$	C, H, N	50	45	59	45	40
								12.5	19	*	*	*
30	H	3-F-C_6H_5	H	50	210–211	$C_{24}H_{27}FClN_3O_2 \cdot 0.75H_2O$	C, H, N	25	29	31	22	18
31	H	H	CH_3	45	260–263	$C_{19}H_{26}ClN_3O_2 \cdot 0.5H_2O$	C, H, N	50	30	36	21	26
32	H	H	CH_2CH_3	52	236–239	$C_{20}H_{28}ClN_3O_2 \cdot 0.5H_2O$	C, H, N	50	26	23	15	*
33	H	CH_3	CH_3	4	145–147	$C_{20}H_{28}ClN_3O_2 \cdot 2.5H_2O$	C, N; H[i]	50	32	*	*	*
34	H	CH_3	CH_2CH_3	31	221–222	$C_{21}H_{30}ClN_3O_2$	C, H, N	50	16	*	*	*
35	CH_3	CH_3	H	34	210–212	$C_{20}H_{28}ClN_3O_2$	C, H, N	50	29	22	*	*
36	CH_3	CH_2CH_3	H	30	237–238	$C_{21}H_{30}ClN_3O_2 \cdot 0.5H_2O$	C, H, N	50	20	*	*	*
37	CH_2CH_3	CH_3	H	20	266–268	$C_{21}H_{30}ClN_3O_2$	C, H, N	50	*	*	*	*

no.	X	R	yield,[a] %	mp, °C[b]	formula	anal.[c]	dose, mg/kg po	% fall in SBP[d] 1 h	2 h	3 h	4 h
38	H	CH_3	45	159–163	$C_{18}H_{26}ClN_3O \cdot 0.5H_2O$	C, H, N	50	30	26	15	26
39	H	CH_2CH_3	23	209–210	$C_{19}H_{28}ClN_3O$	C, H, N	50	23	23	15	*
40	H	CH_2OCH_3	27	179–180	$C_{19}H_{28}ClN_3O_2$	C, H, N	50	20	18	13	26
41	H	C_6H_5	55	199–201	$C_{23}H_{28}ClN_3O \cdot 0.25H_2O$	C, H, N	50	*	27	30	32
42	OCH_3	CH_3	14	275–278	$C_{19}H_{27}N_3O_2 \cdot H_2O$[e]	C, H; N[j]	50	17	*	*	*
43	OCH_3	C_6H_5	8	140–144	$C_{24}H_{30}ClN_3O_2 \cdot 0.5H_2O$	C, H; N[k]	50	30	28	22	*
20		see text	91	indef	$C_{16}H_{25}Cl_2N_3 \cdot H_2O$	C, H, N	50	20	24	*	20

no.	X	A	R	R_1	R_2	yield,[a] %	mp, °C[b]	formula	anal.[c]	dose, mg/kg po	% fall in SBP[d] 1 h	2 h	3 h	4 h
44		CH_2CH_2	H	H	H	37	247–250	$C_{16}H_{23}ClN_2O_2$	C, H, N	12.5	46	35	30	27
										6.25	*	*	*	*
45	4-OCH_3	CH_2CH_2	H	H	H	20	229–231	$C_{17}H_{25}ClN_2O_3 \cdot 0.25H_2O$	C, H, N	12.5	*	*	*	*
46	4-OH	CH_2CH_2	H	H	H	44	135–137	$C_{16}H_{23}ClN_2O_3 \cdot 4H_2O$	C, H, N	25	13	*	11	21

47	4-F	CH_2CH_2	H	H	H	15	249–251	$C_{16}H_{22}ClFN_2O_2 \cdot 0.75H_2O$	C, H, N	12.5	*	*	*	*
48	4-Cl	CH_2CH_2	H	H	H	15	269–270	$C_{16}H_{22}Cl_2N_2O_2 \cdot 0.5H_2O$	C, H, N	12.5	*	*	*	*
49	3,4-OCH_3	CH_2CH_2	H	H	H	56	244–246	$C_{18}H_{27}ClN_2O_4 \cdot 0.25H_2O$	C, H, N	50	*	*	*	*
50		$CHOHCH_2$	H	H	H	43	148–150	$C_{16}H_{22}N_2O_3$[e]	C, H, N	50	*	*	*	*
51		$OCH_2CHOHCH_2$	H	H	H	26	indef	$C_{17}H_{25}ClN_2O_4 \cdot 0.5H_2O$	C, H; N[l]	50	*	18	*	*
52		$OCH_2CH_2CH_2$	H	H	H	13	167–168	$C_{17}H_{25}ClN_2O_3 \cdot 0.5H_2O$	C, H, N	12.5	*	*	*	*
53	4-F	$COCH_2CH_2$	H	H	H	44	206–210	$C_{17}H_{22}FClN_2O_3$	C, H, N	25	27	24	*	20
54	4-F	$COCH_2CH_2CH_2$	H	H	H	25	225–230	$C_{18}H_{24}FClN_2O_3 \cdot 0.5H_2O$	C, H, N	25	50	33	29	30
										12.5	*	*	*	18
55	4-F	$COCH_2CH_2CH_2$	H	CH_3	H	14	196–198	$C_{19}H_{26}FClN_2O_3 \cdot H_2O$	C, H, N	12.5	46	37	25	19
										3.1	26	27	*	*
56	4-F	$COCH_2CH_2CH_2$	H	CH_2CH_3	H	33	210–213	$C_{20}H_{28}FClN_2O_3 \cdot 0.75H_2O$	C, H, N	25	35	31	27	13
57	1,2,3,4-tetrahydronaphth-2-yl		H	H	H	13	155–157	$C_{18}H_{24}N_2O_2 \cdot 0.25H_2O$[e]	C, H; N[m]	50	28	24	23	20
58	see text						228–230	$C_{19}H_{27}ClN_2O_4 \cdot 0.75H_2O$	C, H, N	50	*	*	*	*

											% fall in SBP[d]			
no.	A	R	R_1	R_2	yield,[a] %	mp, °C[b]	formula	anal.[c]	dose, mg/kg po	1 h	2 h	3 h	4 h
59	$CHOHCH_2$[n]	H	H	H	60	177–180	$C_{18}H_{25}ClN_2O_5 \cdot 2H_2O$	C, H, N	25	*	*	*	20
60	$CHOHCH_2$[o]	H	CH_3	H	11	156–160	$C_{19}H_{27}ClN_2O_5 \cdot 0.5H_2O$	C, H, N	25	41	33	17	*
61	$CHOHCH_2$[n]	H	CH_3	H	38	211–213	$C_{19}H_{27}ClN_2O_5$	C, H, N	25	41	36	40	40
									12.5	25	22	*	19
62	$CHOHCH_2$[n]	H	C_6H_5	H	72	203–205	$C_{24}H_{29}ClN_2O_5 \cdot 0.5H_2O$	C, H, N	25	*	25	*	16
63	$CHOHCH_2$[n]	CH_2CH_3	H	H	82	263–266	$C_{20}H_{29}ClN_2O_5 \cdot 0.75H_2O$	C, H, N	50	40	44	36	26
64	$CHOHCH_2$[n]	C_6H_5	H	H	38	171–172	$C_{24}H_{29}ClN_2O_5 \cdot 0.5H_2O$	C, H, N	50	*	*	*	*
65	CH_2	H	H	H	13	164–166	$C_{17}H_{23}ClN_2O_4 \cdot 1.5H_2O$	C, H, N	25	53	49	40	38
									6.25	38	36	21	*
66	CH_2CH_2	H	H	H	48	225–227	$C_{18}H_{25}ClN_2O_4 \cdot 0.5H_2O$	C, H, N	50	44	41	37	31
									25	29	18	8	20
2 (indoramin)									25	27	28	34	30
									12.5	*	*	*	*
prazosin									1.25	26	46	36	31
									0.31	19	31	21	26

[a] Yield refers to the last step in each synthetic sequence. [b] Melting point of HCl salt unless otherwise indicated. [c] Elemental analyses were within 0.4% of theory unless otherwise noted. [d] Four rats per dosage group. Percentage falls in systolic blood pressure were recorded at the indicated hour after dosing on the second day of dosing. Systolic pressures in the controls started at about 200 mmHg and varied over the range of 180–220 mmHg during the 4-h measurement period. Values in the table are statistically significant ($p \leq 0.05$) relative to control values; asterisks indicate nonsignificance ($p \geq 0.05$). [e] Free base was analyzed and tested. [f] Percent fall after 8 h. [g] C: calcd, 62.71; found, 63.87. [h] N: calcd, 10.86; found, 11.42. [i] H: calcd, 7.89; found, 7.44. [j] N: calcd, 12.09; found, 13.06. [k] N: calcd, 9.61; found, 8.95. [l] N: calcd, 7.66; found, 6.76. [m] N: calcd, 9.19; found, 8.72. [n] Erythro isomer. [o] Erythro-threo mixture (1:1).

Table II. α_1- and α_2-Adrenoceptor Affinities

compd	$-\log K_I^a$ (α_1) ± SE of mean	n^b	$-\log K_I^a$ (α_2) ± SE of mean	n
prazosin	9.22 ± 0.19	3	6.19 ± 0.33	3
indoramin	7.61 ± 0.35	3	4.92 ± 0.29	3
21	7.41 ± 0.22	3	6.20 ± 0.18	3

[a] Determined in rat cerebral cortex membranes with [^{3}H]prazosin and [^{3}H]yohimbine to label α_1 and α_2 adrenoceptors, respectively. [b] SE = standard error; n = number of determinations.

4.00 (ddd, J = 7.5, 3, and 3 Hz, 1 H), 4.15 (dd, J = 12 and 7.5 Hz, 1 H), 4.32 (s, 1 H), 4.42 (dd, J = 12 and 3 Hz, 1 H), 4.80 (OH), 6.85 (m, 4 H), 7.28 (m, 3 H), 7.42 (m, 2 H).

The HCl salt was crystallized from 2-propanol–ether.

1-(2-Indol-3-ylethyl)-4-methoxy-4-(benzamidomethyl)-piperidine Hydrochloride (43). A solution of 10 g (40 mmol) of epoxide **6** (R = H) and 8.5 g (130 mmol) of NaN_3 in 50 mL of dioxane and 15 mL of water was stirred at 85 °C for 18 h. The mixture was concentrated and partitioned between ether and water. The ether was washed with brine and dried (Na_2SO_4). Evaporation of the ether gave 11.3 g (96%) of crude **15** as a semisolid.

Sodium hydride (2.0 g of 50% in oil, 43 mmol) was washed with hexane, and 50 mL of DMF was added. A solution of 11 g (38 mmol) of **14** in 50 mL of DMF was added, and the resulting solution was stirred for 15 min at room temperature. Methyl iodide (3 mL) was slowly added, and stirring was continued for 2 h. The mixture was poured into water and extracted with ethyl acetate. The ethyl acetate was washed with water and brine and then dried (Na_2SO_4). Evaporation left 10 g (87%) of **16** as an oil. A solution of 2.9 g (9.5 mmol) of **16** and 0.2 g of 10% palladium on carbon in 50 mL of EtOH was hydrogenated at 40 psi for 45 min. The mixture was filtered and the filtrate was evaporated to yield 1.6 g of crude **17**. Amine **17** (6.0 g, 22 mmol) in 100 mL of ethyl acetate and 80 mL of water containing 10 g of K_2CO_3 was treated with 5 mL of benzoyl chloride while the mixture was stirred in an ice bath. The ethyl acetate was dried (Na_2SO_4) and evaporated. Chromatography of the residue on silica gel (ether) gave 5.0 g (60%) of **18** as an oil.

A solution of 4.2 g (11 mmol) of **18** in 10 mL of acetic acid was treated with 60 mL of 1 M HBr in acetic acid. The mixture was stirred for 2 h and then diluted with ether. The solvent was decanted off, and the residue was recrystallized from 2-propanol to afford 3.5 g (97%) of the HBr salt (R = phenyl).

A solution of 3.3 g (10.6 mmol) of the HBr salt and 2.6 g (11.7 mmol) of 2-indolylethyl bromide in 70 mL of DMF and 5 mL of triethylamine was stirred at 70 °C for 20 h. The mixture was added to water and extracted with CH_2Cl_2. Evaporation of solvent and chromatography of the residue on silica gel (ethyl acetate) gave the free base **43**: mp 85–88 °C; IR (KBr) 3400, 1640, 1525 cm^{-1}; NMR ($CDCl_3$) δ 1.80–2.15 (m, 4 H), 2.60–3.35 (m, 8 H), 3.26 (s, 3 H), 3.60 (br t, 2 H), 7.00–8.00 (m, 10 H), 3.00 (exchangeable); MS m/e 391 (M^+), 359, 261, 229, 130, 105.

Conversion to the HCl salt with MeOH–HCl and precipitation with ether afforded 0.36 g (8%) of **43**·HCl.

1-(2-Indol-3-ylethyl)-4-(benzamidomethyl)piperidine Hydrochloride (41). Isonipecotamide (8.0 g, 62.5 mmol) and 5.0 g (22 mmol) of 2-indolylethyl bromide in 60 mL of DMF and 8 mL of triethylamine were stirred at 75 °C for 12 h. The mixture was poured into water and extracted with CH_2Cl_2. The CH_2Cl_2 extract was evaporated, and the residue was triturated with ethyl acetate to give 5.9 g (98%) of crude **19**. This material was added in several portions to 3.3 g (87 mmol) of lithium aluminum hydride in 300 mL of THF, and the mixture was heated to reflux for 1.5 h. Water (3 mL), 4 mL of 10% NaOH solution, and 11 mL of water were added, and the mixture was filtered. Evaporation of the filtrate left a residue, which was triturated with ether to afford 5.1 g (91%) of free base **20**, mp 123–125 °C.

Amine **20** (2.0 g, 7.8 mmol) in a mixture of 25 mL of ethyl acetate and 20 mL of water containing 2.5 g of K_2CO_3 in an ice bath was treated with 1 mL of benzoyl chloride. The mixture was stirred for 1 h, and the ethyl acetate layer was dried (Na_2SO_4) and evaporated. Trituration of the residue with ether afforded the free base **41**: mp 121–125 °C; IR (KBr) 3500–3200, 1635, 1530 cm^{-1}; NMR ($CDCl_3$) δ 1.50–2.30 (m, 5 H), 2.70–3.40 (m, 10 H), 6.85–7.85 (m, 11 H), 8.40 (1 H); MS, m/e 361 (M^+), 231, 144, 130, 105, 77.

The base was dissolved in MeOH, and the solution was acidified with HCl. Ether was added to precipitate the HCl salt, which was recrystallized from 2-propanol to give 1.7 g (55%) of **41**·HCl.

Antihypertensive Screen. After an initial training period, 24 male, Okamoto-Aoki strain, spontaneously hypertensive rats (Taconic Farms, Germantown, NY) were distributed into six groups of four animals with approximately equal mean systolic blood pressures. The six groups were studied concurrently in a 2-day procedure. Test compounds were randomly assigned to each group. Five groups received test substances, and one control group received vehicle only. On two consecutive mornings, a group of four rats was orally dosed with a test substance that had been dissolved or suspended in water at concentrations such that 0.1 mL of solution was administered per 10 g of body weight. Immediately after dosing on day 2, all 24 rats were put in restrainers and then into a heated chamber (30.0 ± 1.0 °C) for 4 h. Systolic blood pressures (tail cuff) were recorded with photoelectric transducers at 1, 2, 3, and 4 h after drug administration. The coccygeal arteries of the rats were simultaneously occluded by inflated tail cuffs that were automatically inflated to 300 mmHg and then deflated. Tail pulses were simultaneously recorded, along with a pressure curve, on a recorder. Four consecutive (at 3-s intervals) traces were recorded for each rat at each hour after dosing. The systolic pressure was considered to be the pressure at the appearance of the first pulse. The mean systolic pressure of each rat at each observation time in both drug-treated and control groups was calculated. Systolic pressures in the controls varied over the range 180 to 220 mmHg during the 4-h measurement period. The mean values of the respective drug-treated and control groups were then compared by using a 1-tail Student's t test. Statistical significance was considered to be $p \leq 0.05$.

Ligand Binding Studies. Binding assays were performed with washed rat cerebral cortex membranes. [^{3}H]Prazosin[11] and [^{3}H]yohimbine[12] were used to label α_1 and α_2 adrenoceptors, respectively. Membranes were prepared by homogenization of tissue in 50 mM Tris-HCl buffer (pH 7.8) and centrifugation at 48000g for 15 min. The membrane pellet was washed 3 times with the Tris-HCl buffer before use. Competition experiments were performed with 100-μL aliquots of the membrane suspension (2.5 mg/mL of protein) incubated with the radioligand and competing drug in a volume of 0.25 mL. After a 30-min equilibration period at 22 °C, the incubation was terminated by addition of 1.5 mL of ice-cold buffer, followed by filtration and rapid washing with Tris-HCl buffer (3 × 5 mL). Norepinephrine (200 μmol) was used to define nonspecific binding in both studies. Specific binding of [^{3}H]yohimbine (2.0 nmol) and [^{3}H]prazosin (0.6 nmol) represented 60–70% and 80–90%, respectively, of the total binding. For the competition experiments, an IC_{50} value (the concentration of compound inhibiting specific binding of the radioligand by 50%) was calculated for each compound, and the K_I value was determined by the expression $K_I = IC_{50}/(1 + [L]/K_D)$ where [L] represents the concentration of radioligand in the assay, and K_D is the affinity constant for the radioligand.[13]

Acknowledgment. We thank Drs. J. Muchowski and A. Kluge for much helpful discussion. The assistance of Dr. M. Maddox and Syntex Analytical Research is also gratefully acknowledged.

Registry No. **6** (R = H), 77211-75-7; **7** (R = R^1 = H), 85151-16-2; **7** (R = H; R$_1$ = Ph), 77211-76-8; **8** (R = R$_1$ = R$_2$ = H; X = Cl), 85151-17-3; **8** (R = R$_2$ = H; R$_1$ = Ph; X = Cl), 85151-18-4; **9** (R = R$_1$ = R$_2$ = H), 84243-24-3; **10**·HBr (R = R$_1$ = R$_2$ = H), 83090-46-4; **10** (R = R$_2$ = H; R$_1$ = Ph), 85151-19-5; **13**, 78339-59-0; **14**, 83917-83-3; **15**, 85151-20-8; **16**, 85151-21-9; **17**, 85151-22-0; **18** (R = Ph), 85151-23-1; **18**·HBr (R = Ph), 85151-24-2; **19**, 4077-20-7; **20**, 85151-25-3; **20**·HCl, 85151-26-4; **21**, 83090-47-5; **21**·HCl, 85151-27-5; **22**, 83090-39-5; **23**, 83090-40-8; **23**·HCl, 85151-28-6; **24**, 85151-29-7; **24**·HCl, 85151-30-0; **25**, 83090-13-5; **25**·HCl, 85151-31-1; **26**, 83090-14-6; **26**·HCl, 85151-32-2; **27**, 83090-17-9; **27**·HCl, 85151-33-3; **28**, 85151-34-4; **28**·HCl, 85151-35-5; **29**, 85151-36-6; **29**·HCl, 85151-37-7; **30**, 85151-38-8; **30**·HCl, 85151-39-9; **31**, 83090-19-1; **31**·HCl, 85151-40-2; **32**, 83090-20-4; **32**·HCl, 85151-41-3; **33**, 83090-21-5; **33**·HCl, 85151-42-4; **34**,

83090-22-6; **34**·HCl, 85151-43-5; **35**, 83090-18-0; **35**·HCl, 85151-44-6; **36**, 83090-15-7; **36**·HCl, 85151-45-7; **37**, 83090-16-8; **37**·HCl, 85151-46-8; **38**, 85151-47-9; **38**·HCl, 85151-48-0; **39**, 85151-49-1; **39**·HCl, 85151-50-4; **40**, 85151-51-5; **40**·HCl, 85151-52-6; **41**, 85151-53-7; **41**·HCl, 85151-54-8; **42**, 85151-55-9; **43**, 85151-56-0; **43**·HCl, 85220-62-8; **44**, 84243-26-5; **44**·HCl, 84269-54-5; **45**, 85151-57-1; **45**·HCl, 84243-27-6; **46**, 85151-58-2; **46**·HCl, 84243-29-8; **47**, 85151-59-3; **47**·HCl, 84243-31-2; **48**, 85151-60-6; **48**·HCl, 84243-30-1; **49**, 85151-61-7; **49**·HCl, 84243-28-7; **20**, 85151-62-8; **21**, 85151-63-9; **51**·HCl, 85151-64-0; **52**, 85151-65-1; **52**·HCl, 85151-66-2; **53**, 85151-67-3; **53**·HCl, 84243-36-7; **54**, 84243-32-3; **54**·HCl, 84243-33-4; **55**, 85151-68-4; **55**·HCl, 84243-34-5; **56**, 85151-69-5; **56**·HCl, 84243-35-6; **57**, 85151-70-8; **58**, 85151-71-9; **58**·HCl, 85151-72-0; **59**, 85151-73-1; **59**·HCl, 85151-74-2; **60**, 85151-75-3; **60**·HCl, 85151-76-4; **61**, 85151-77-5; **61**·HCl, 85151-78-6; **62**, 85151-79-7; **62**·HCl, 85151-80-0; **63**, 85151-81-1; **63**·HCl, 85151-82-2; **64**, 85151-83-3; **64**·HCl, 85151-84-4; **65**, 85151-85-5; **65**·HCl, 84033-74-9; **66**, 84033-72-7; **66**·HCl, 84033-73-8; 2-indolylethyl bromide, 3389-21-7; aniline, 62-53-3; benzoyl chloride, 98-88-4; isonipecotamide, 1453-82-3.

Oxidation of Uric Acid. 4. Synthesis, Structure, and Diabetogenic Action of 5-Imino-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione Salts and Their Alloxan-Like Covalent Adducts[1]

Mirko Poje,*[†] Boris Ročić,[‡] Milan Sikirica,[§] Ivan Vicković,[§] and Milenko Bruvo[§]

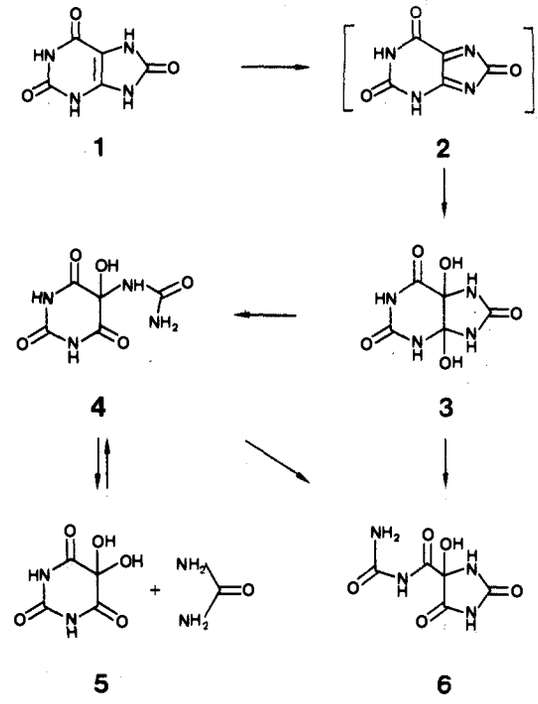
Laboratory of Organic Chemistry and Laboratory of General and Inorganic Chemistry, Faculty of Science, and Institute for Diabetes, Endocrinology, and Metabolic Diseases "Vuk Vrhovac", Medical Faculty, University of Zagreb, 41000 Zagreb, Yugoslavia. *Received July 19, 1982*

Three synthetic routes to salts of 5-amino-5-hydroxy-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione (**10**) are described. The key reactions involved acid-catalyzed cleavage of 5-amino-5-ureido-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione (**7**), conversion of uramil (**8**) to dehydrouramil (**9**) and subsequent hydration, and the condensation of alloxan (**5**) with ammonium salts. The carbinol ammonium salt structure **10a** was unambiguously established by X-ray crystallography. New alloxan-like compounds **7**, **9**, and **10** were evaluated for diabetogenic activity in rats. Compound **7** was inactive, whereas compounds **9** and **10** showed the highest activity comparable to that of streptozotocin (**12**).

The discovery of alloxan diabetes has led to early suggestions that a substance biogenetically related to uric acid (**1**) may have an alloxan-like action on the β cells of islets and, thus, produce diabetes.[2] The question of whether dehydrouric acid (**2**) can be a transient intermediate in uricolysis has aroused recent interest.[3] Mechanistic, as well as practical, considerations make the still unavailable quinonoid system **2** an interesting synthetic target. Although considerable work on the oxidative breakdown of **1** has been described previously by Biltz and his school,[4] the problem of the constitution of intermediates has presented paradoxes that caused much confusion in this field, and little is known about the chemical and biological properties of these intermediates. Our own interest in the oxidation of uric acid (**1**) originated with the intent to explore the biological effects of its alloxan-like derivatives. The structural elucidations of tetrahedral adduct **3** and derivatives **4** and **6**, resulting from regiospecific cleavages of ortho acid aminal array,[5,6] have helped to unravel the chemistry and biological effects of alloxan-like derivatives of **1**. We have examined compounds **3** and **4** to test the concepts of Brückmann and Wertheimer[7] and to determine whether there might be other structural features that correlate with diabetogenic activity. The highly specific diabetogenic action of these compounds has challenged accepted structure–activity relationships.[1] The possible relationship between the quinonoid structure **2**, or adducts derived therefrom, and biological response gave an impetus to further explore the possibility of finding new diabetogenic compounds related to uric acid (**1**).

The essence of our plan germinated from a claim that ammonium salts exerted an apparently specific potentiating action on the diabetogenic effect of alloxan.[7] It appeared therefore, that it would be interesting to study

Scheme I. Oxidative Degradation of Uric Acid (**1**)



alloxan-like systems that incorporate an amino function. To test these ideas, we required a straightforward synthesis

[†] Laboratory of Organic Chemistry.
[‡] Laboratory of General and Inorganic Chemistry.
[§] Institute for Diabetes, Endocrinology, and Metabolic Diseases.

(1) Part 3 of this series: Poje, M.; Ročić, B. *Experientia* **1980**, *36*, 78.
(2) (a) Lazarow, A. *Physiol. Rev.* **1949**, *29*, 48. (b) Griffiths, M. *J. Biol. Chem.* **1950**, *184*, 289, claimed that the administration of **1** in glutathione-depleted rabbits caused diabetes. (c) Reports have been published that a number of enzymes are capable of catalyzing the oxidation of **1** to **5**: Soberon, G.; Cohen, P. P. *Arch. Biochem. Biophys.* **1963**, *103*, 331, and references cited therein.

0022-2623/83/1826-0861$01.50/0