triamines[6] reported previously. The lack of antimalarial activity is difficult to reconcile. Possibly the presence of nitrogen in position 5 still allows the formation and interconversion of one-carbon units in reduced forms of these compounds analogous to various 5,6,7,8-tetrahydrofolate coenzymes, as opposed to the 2,4-diaminoquinazoline antimetabolites in which the absence of N-5 could preclude such changes, thus inhibiting the critical biochemical pathway.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus (capillary method) and are corrected. The progress of the reactions was followed by TLC using silica gel plates (Eastman) and a solvent mixture of MeOH–EtOAc–Et_3N, 25:75:1. Satisfactory infrared spectra were obtained for all compounds.

Preparation of 6-[(Phenyl and naphthyl)thio]-2,4-pteridinediamines (Table I; 1–7). The preparation of 6-[(2,4,5-trichlorophenyl)thio]-2,4-pteridinediamine (Table I; 1) is described as an example. A mixture of 1.0 g (0.005 mol) of 6-chloro-2,4-pteridinediamine, 1.24 g (0.006 mol) of 2,4,5-trichlorobenzenethiol, and 10 g of dimethyl sulfone was stirred at 192–200 °C for 20 min, cooled slightly, and poured into H_2O. The warm mixture was made basic with 50% aqueous NaOH and filtered to collect the precipitate that had formed. The filter cake was washed with H_2O and then with 20 mL of acetone and was then recrystallized from DMF to give 0.97 g (51%) of the product, mp >300 °C.

6-[[(4-Chlorophenyl)methyl]thio]-2,4-pteridinediamine (Scheme I; 8). A mixture of 3 g (0.0153 mol) of 6-chloro-2,4-pteridinediamine, 2.45 g (0.0155 mol) of 4-chlorobenzenemethanethiol, and 22 g of dimethyl sulfone was heated at 195 °C (external temperature) for 0.5 h. The temperature of the reaction mixture rose to 203 °C. The mixture was allowed to cool to 130 °C, poured into H_2O, and filtered. The filter cake was washed successively with acetone, 1 N NaOH, H_2O, and acetone again. The dark material was then extracted with EtOH continuously in a Soxhlet extractor for 25 h. The extract was evaporated in vacuo to give 0.5 g of crude product. Recrystallization from DMF gave 0.32 g (6.6%) of the title compound, mp 275–277 °C dec. Anal. ($C_{13}H_{11}ClN_6S$) C, H, N.

***N',N''*-[6-[(3,4-Dichlorophenyl)thio]-2,4-pteridinediyl]-bis[*N,N*-dimethylformamidine] (Scheme I; 9).** A mixture of 0.73 g (0.002 mol) of 6-[(3,4-dichlorophenyl)thio]-2,4-pteridinediamine, 5.14 g (0.04 mol) of *N,N*-dimethylformamide dimethyl acetal, and 15 mL of DMF was stirred at room temperature for 20 h, chilled, and filtered to give 0.80 g (89%) of the product, mp 231–234 °C. Anal. ($C_{18}H_{18}Cl_2N_8S$) C, H, N.

Acknowledgment. The authors are indebted to Drs. M. W. Fisher and C. L. Heifitz of Warner-Lambert Co. for the antibacterial studies. We also thank C. E. Childs and associates for the microanalyses and Dr. J. M. Vandenbelt and co-workers for the determination of spectral data.

Diuretic Agents Related to Indapamide. 1. Synthesis and Activity of 1-Substituted 2-(4-Chloro-3-sulfamoylbenzamido)isoindolines

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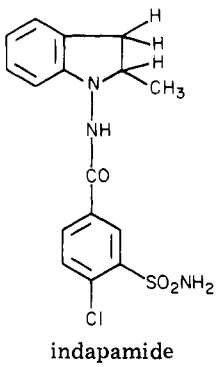
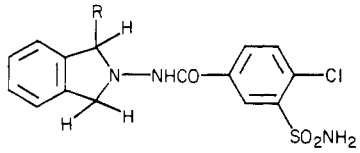
The synthesis of isoindoline analogues of indapamide is described. These substances (**4a,c,d**) were tested for diuretic activity, and **4a** was found to be comparable in potency to indapamide.

Indapamide is a diuretic agent widely used in the therapy of hypertension.[1] Comparing its structure to that of clopamide and other 4-chloro-3-sulfamoylbenzhydrazides having diuretic properties,[2] we considered the possibility that the indoline moiety of indapamide might not be essential for drug–receptor interaction. We therefore synthesized **4a** to evaluate its diuretic activity.



indapamide



4a, R = CH_3
c, R = CH_2OH
d, R = COOH

The two potential metabolites of **4a**, namely **4c** (R = CH_2OH) and **4d** (R = COOH), were also synthesized and tested.

Chemistry. The starting material for the synthesis of **4a** was the unknown 1-methyl-2-aminoisoindoline (**3a**), which was prepared by condensing the dibromide **1a**[3] with *tert*-butyl carbazate in DMF to give the carbamate **2a**; **3a**·HCl was recovered when **2a** was treated with dilute HCl. The overall yield was 86%.

Final condensation of **3a** with 4-chloro-3-sulfamoylbenzoyl chloride[4] yielded 67% of **4a**. This was accom-

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(1) (a) W. P. Leary, A. C. Asmal, and P. Samuel, *Curr. Ther. Res. Clin. Exp.*, **15**, 571 (1973); (b) W. P. Leary, A. C. Asmal, Y. K. Seedat, and P. Samuel, *S. Afr. Med. J.*, **48**, 119 (1974); (c) G. Gragnoli, F. Puccetti, R. Nami, I. Tanganelli, F. Favilli, and E. Betti, *Minerva Med.*, **68**, 749 (1977); (d) P. Turner, G. N. Volans, and H. J. Rogers, *Curr. Med. Res. Opin.*, **4**, 596 (1977).
(2) E. Jucker, A. Lindenmann, E. Schenker, E. Flückiger, and M. Taeschler *Arzneim.-Forsch.*, **13**, 269 (1963).
(3) G. Pifferi, R. Monguzzi, and P. Consonni, *Farmaco, Ed. Sci.*, **27**, 30 (1972).
(4) Parke-Davis and Co., British Patent 915 259 (1963); *Chem. Abstr.*, **61**, 6962 (1961).

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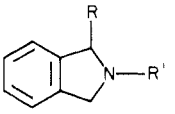
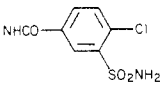
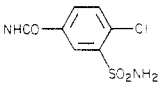
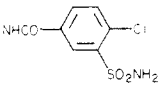
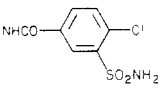
Table I. 1,2-Disubstituted Isoindolines[a]



compd	R	R'	yield, %	mp, °C	purifn proced[b]	formula[c]
2a	CH_3	$NHCO_2C(CH_3)_3$	77	144-145	I	$C_{14}H_{20}N_2O_2$
2b	CO_2CH_3	$NHCO_2C(CH_3)_3$	81	160-162[d]	II	$C_{15}H_{20}N_2O_4$
3a	CH_3	NH_2	86	148-150	III	$C_9H_{12}N_2 \cdot HCl$
3b	CO_2CH_3	NH_2	76	151-153	III	$C_{10}H_{12}N_2O_2 \cdot HCl$
4a	CH_3		67	213-215	IV	$C_{16}H_{16}N_3O_3ClS$
4b	CO_2CH_3		70	156-157	IV	$C_{17}H_{16}N_3O_5ClS$
4c	CH_2OH		75	220-222	V	$C_{16}H_{16}N_3O_4ClS$
4d	COOH		80	154-156	VI	$C_{16}H_{14}N_3O_5ClS \cdot H_2O$

[a] All compounds exhibited IR and NMR spectra consistent with the assigned structures. [b] I, trituration with *n*-pentane; II, chromatography on silica gel; III, recrystallization from EtOH-Et_2O; IV, recrystallization from EtOH; V, trituration with Me_2CO; VI, precipitation from NaOH solution with 2 N HCl. [c] All compounds analyzed for C, H, N, and, where present, Cl or S within ±0.4% of the calculated values. [d] Boiling points were determined at 760 torr.

plished by modifying a procedure reported for the synthesis of indapamide.[5] The analogues **4c** and **4d** were obtained by $LiBH_4$ reduction and by alkaline hydrolysis, respectively, of the 1-carbomethoxy derivative **4b**. The latter was, in turn, synthesized from **1b**[6] by the route followed for **4a**.

The synthetic sequences are outlined in Scheme I. The physical data of all compounds synthesized are collected in Table I.

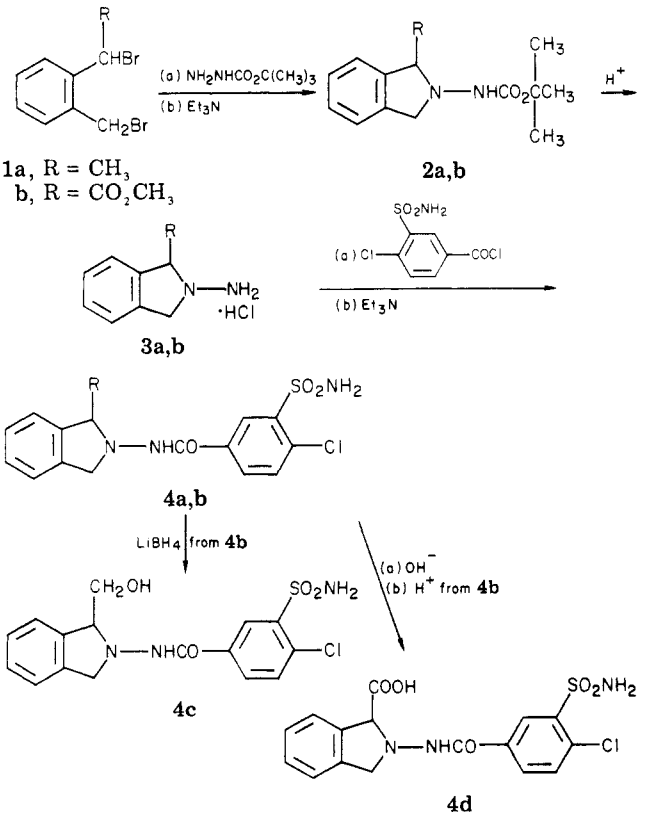
Pharmacology. Compounds **4a,c,d** were screened for diuretic activity with respect to indapamide, and the effects were evaluated on spontaneous diuresis after single (A-1) and repeated (A-2) administration, as well as during water diuresis (B).

As shown in Table II, there was no statistically significant change in the total volume of urine excreted under the action of each agent tested (protocol A-1). However, the indapamide isomer **4a**, as well as indapamide, significantly enhanced Na^+ excretion and exhibited a Na^+/K^+ ratio significantly higher than controls.

Protocol A-2 established that both water and salt excretion were highest with **4a**, with which the Na^+/K^+ ratio was also significantly higher than with indapamide.

Table III reports the data obtained by protocol B. All compounds caused an increase of urine volume, whereas only **4a** and **4c** resulted in higher electrolyte excretion. Although the quantitative effect caused by **4a** was not significantly different from that of indapamide, the Na^+/K^+ ratio was higher for the former compound; although they are of borderline significance, these results are consistent with those obtained by protocol A-2.

Scheme I



Discussion

The finding of major interest in the present study was the demonstration that the indapamide isomer **4a**, which differs from the model compound in having a 1-methylisoindoline rather than a 2-methylindoline moiety in its structure, exhibits high diuretic and natriuretic activity.

The observation that **4a**, although comparable to indapamide for effects on urine volume and Na^+ excretion, induced less K^+ loss with a higher Na^+/K^+ ratio could be of clinical importance in the treatment of hypertension, since indapamide may cause hypokalemia.[7]

(5) L. Beregi, P. Hugon, and M. Laubie, French Demande 2 003 311 (1969); *Chem. Abstr.*, **72**, 100 500*t* (1970).
(6) G. Cignarella, R. Cerri, G. Grella, and P. Sanna, *Gazz. Chim. Ital.*, **106**, 65 (1976).

Table II. Effects of Single Administration (A-1) and Repeated Administration (A-2) of Indapamide and of Some Isoindoline Derivatives on Water and Electrolyte Excretion by Rats[a]

compd	N A-1	N A-2	urine vol, mL A-1	urine vol, mL A-2	urine Na^+, mequiv A-1	urine Na^+, mequiv A-2	urine K^+, mequiv A-1	urine K^+, mequiv A-2	urine Na^+/K^+ ratio A-1	urine Na^+/K^+ ratio A-2
4a	6	6	11.67 ± 0.833	16.17 ± 0.946[b]	2.193 ± 0.130[b]	3.058 ± 0.343[b]	1.976 ± 0.172	2.560 ± 0.324	1.128 ± 0.0476[b]	1.2204 ± 0.0450[b,c]
4c	6	11	11.17 ± 2.189	11.45 ± 2.069	1.622 ± 0.197	1.414 ± 0.1683	1.486 ± 0.162	1.512 ± 0.1827	1.084 ± 0.0460	0.94307 ± 0.0340
4d	6	11	9.36 ± 1.430	9.36 ± 1.022	1.590 ± 0.235	2.199 ± 0.1651	1.569 ± 0.287	2.075 ± 0.1695	0.984 ± 0.0835	1.0809 ± 0.0435
indapamide	6	6	12.83 ± 2.455	16.67 ± 2.044[b]	2.563 ± 0.4046[b]	2.947 ± 0.3809[b]	2.198 ± 0.330	2.780 ± 0.3196[b]	1.143 ± 0.0435[b]	1.0526 ± 0.0417
drug vehicle	12	6	8.25 ± 1.762	9.83 ± 2.00	1.630 ± 0.174	1.813 ± 0.2471	1.633 ± 0.172	1.793 ± 0.2535	0.951 ± 0.0463	1.0125 ± 0.0502

[a] The table reports the cumulative amounts (means plus or minus standard error) excreted during 18 h following the administration of the test drug (protocols A-1 and A-2). By protocol A-1, each drug tested (5 mg/kg) was administered once per os at time 0 when the animals were placed in metabolic cages for 18-h urine collection. With protocol A-2, oral administration occurred twice, first at time 0 and then 7 h later. Controls were treated with drug solvent (2 mL/kg of body weight) according to the same time schedule. *N* indicates the number of animals. [b] $p < 0.05$ for statistical comparison with the control group. [c] $p < 0.05$ for statistical testing with respect to indapamide group.

A marked decrease in activity was observed in compounds **4c** and **4d**, in which the methyl group of **4a** is substituted by hydrophilic CH_2OH and COOH groups. This results in changes of physicochemical properties, such as pK_a and partition coefficient. In turn, the latter could interfere with absorption and distribution of these compounds.

An additional relevant finding is that the indolinic moiety of indapamide can be replaced by an isoindoline one, without loss of activity; this prompted investigations on isoindoline derivatives of indapamide, which are presently in progress.

Experimental Section

Chemistry. Melting points, determined by a Kofler apparatus, and boiling points are uncorrected. Elemental analyses were performed by the Microanalyses Laboratory of the Institute of Pharmaceutical Chemistry, University of Padua. The IR spectra were obtained with a Perkin-Elmer 297 spectrophotometer and NMR spectra were recorded on a Hitachi Perkin-Elmer R-24 spectrometer using Me_4Si as internal standard.

1,2-Disubstituted Isoindolines (Table I). 1-Methyl-2-[(*tert*-butyloxycarbonyl)amino]isoindoline (2a). To a solution of 8 g (0.0287 mol) of **1a**[3] and 7.14 g (0.0287 mol) of *tert*-butyl carbazate in 25 mL of DMF warmed to 50 °C was added dropwise 9.6 mL (0.069 mol) of triethylamine, while keeping the temperature at 50–60 °C. The mixture was stirred at room temperature for 3 h; H_2O was then added to a final volume of 100 mL, and stirring continued for an additional 60 min. The precipitated solid was collected by filtration, washed with H_2O, and dried: 6.01 g of crude **2a** was obtained. Anal. ($C_{14}H_{20}N_2O_2$) C, H, N.

1-Carbomethoxy-2-[(*tert*-butylcarbonyl)amino]isoindoline (2b). To a solution of 20 g (0.062 mol) of **1b**[6] and 8.21 g (0.062 mol) of *tert*-butyl carbazate in 60 mL of DMF warmed to 50 °C was added 18.9 g (0.136 mol) of triethylamine while keeping the temperature at 50–60 °C. The reaction mixture was then stirred at room temperature for 3 h; 200 mL of H_2O was added. Subsequently, extraction with Et_2O was performed. The combined extracts were dried (Na_2SO_4), and the solvent was removed under reduced pressure to give 17 g of **2b** as a yellowish oil. Anal. ($C_{15}H_{20}N_2O_4$) C, H, N.

1-Methyl-2-aminoisoindoline Hydrochloride (3a). 2a (6 g, 0.024 mol) was added in portions at room temperature to 7 mL of concentrated HCl. The mixture was stirred for 90 min and then concentrated to dryness by a combination of reduced pressure and gentle heating (60–65 °C), leading to 4.51 g of crude **3a**. Anal. ($C_9H_{13}N_2Cl$) C, H, N, Cl.

1-Carbomethoxy-2-aminoisoindoline Hydrochloride (3b). A solution of 13.96 g (0.0536 mol) of **2b** in MeOH (20 mL) was added under continuous stirring to 70 mL of saturated dry HCl–MeOH. The resulting solution was stirred for an additional 60 min and processed as described for **3a** to yield 9.4 g of **3b**. Anal. ($C_{10}H_{13}N_2O_2Cl$) C, H, N, Cl.

1-Methyl-2-(4-chloro-3-sulfamoylbenzamido)isoindoline (4a). The procedure reported for indapamide was modified as follows.[5] 2.25 mL (0.0162 mol) of triethylamine was added twice at 30-min intervals to a stirred suspension of 2.5 g (0.0135 mol) of **3a** in dry THF (25 mL). A solution of 3.44 g (0.0135 mol) of 4-chloro-3-sulfamoylbenzoyl chloride[4] in 70 mL of dry THF was then added. After the solution was stirred at room temperature for 20 h, the triethylamine hydrochloride was removed by filtration, and the filtrate was concentrated to dryness under reduced pressure to yield 3.95 g of crude **4a**. Anal. ($C_{16}H_{16}N_3O_3ClS$) C, H, N, Cl, S.

1-Carbomethoxy-2-(4-chloro-3-sulfamoylbenzamido)isoindoline (4b). Following the procedure described for **4a**, a mixture of 1.86 g (0.008 mol) of **3b**, 2.05 g (0.008 mol) of 4-chloro-3-sulfamoylbenzoyl chloride, and 2.7 mL (0.0194 mol) of triethylamine in 30 mL of dry THF resulted in the formation of 3.9 g of **4b**. Anal. ($C_{17}H_{16}N_3O_5ClS$) C, H, N, Cl, S.

(7) (a) J. L. Imbs and J. Schwartz, *Nouv. Presse Med.*, 7, 2477 (1978); (b) O. Rodat and J. P. Hamelin, *ibid.*, 7, 3054 (1978); (c) R. Thill, *ibid.*, 7, 4154 (1978).

Table III. Effects of Indapamide and of Some Isoindoline Derivatives on Water and Electrolyte Excretion by Water-Loaded Rats[a]

compd	urine vol as a % of oral water load	urine Na^+, mequiv	urine K^+, mequiv	urine Na^+/K^+ ratio
4a	127.0 ± 4.457[b]	0.6927 ± 0.0504[b]	0.2398 ± 0.0278[b]	3.110 ± 0.3012[b]
4c	106.0 ± 7.199[b]	0.2629 ± 0.0452[b]	0.1657 ± 0.0213[b]	1.638 ± 0.2337[b]
4d	87.1 ± 5.453[b]	0.1090 ± 0.0302	0.1305 ± 0.0264	0.855 ± 0.1953
indapamide	130.6 ± 5.706[b]	0.7052 ± 0.0619[b]	0.2686 ± 0.0289[b]	2.757 ± 0.2452[b]
drug vehicle	68.3 ± 6.493	0.0780 ± 0.0180	0.1031 ± 0.0167	0.738 ± 0.0868

[a] The data refer to protocol B. Mean plus or minus standard errors are reported for the cumulative urinary excretion measured in the 4 h following oral administration of the drug tested. The data were obtained on five male and five female rats. All drugs were tested at a dose of 5 mg/kg. [b] $p < 0.05$ for statistical testing with respect to control group.

1-(Hydroxymethyl)-2-(4-chloro-3-sulfamoylbenzamido)-isoindoline (4c). A solution of 5.64 g (0.0137 mol) of **4b** in dry THF (80 mL) was added dropwise to a stirred suspension of 0.6 g (0.027 mol) of $LiBH_4$ in 50 mL of dry THF at 0 °C. The stirred mixture was refluxed for 2 h and then cooled to 0 °C during the slow addition of 35 mL of H_2O. The inorganic precipitate was filtered and thoroughly washed with THF. The filtrates were collected, dried with Na_2SO_4, and concentrated to dryness in vacuo to give 4 g of crude **4c**. Anal. ($C_{16}H_{16}N_3O_4ClS$) C, H, N, Cl, S.

1-Carboxy-2-(4-chloro-3-sulfamoylbenzamido)isoindoline (4d). A solution of 6 g (0.0146 mol) of **4b** in 60 mL of 1 N NaOH (1:1 H_2O–EtOH) was stirred for 2 h at room temperature. After the EtOH was removed under reduced pressure, the aqueous layer was acidified with 2 N HCl. The resulting precipitate was then collected by filtration, yielding 4.65 g of crude **4d**. Anal. ($C_{16}H_{14}N_3O_5ClS·H_2O$) C, H, N, Cl, S.

Pharmacology. Experiments were performed on male (200–250 g) and female (160–220 g) Wistar rats, after 16 h of fasting. Three different protocols were used. In protocol A-1, male animals were fed by gastric tubing with a dose of 5 mg/kg of the agent tested in 1% saline. In protocol A-2, an identical dose was given, again 7 h later. Urine was collected for 18 h following drug administration. Protocol B was performed on both male and female rats. It consisted of an oral load of 50 mL/kg of tap water containing the drug to be tested. The urine was collected for 4 h following loading. Control animals received only the drug solvent. Sodium and potassium were measured by a Radiometer KBH flame photometer. Means and standard errors were calculated. Differences between means were tested for significance by Student's t test.

Nonsteroidal Antiinflammatory Agents. 3.[1] Synthesis of the Positional Isomers of 4′-Chloro-5-methoxy-3-biphenylylacetic Acid and Their Antiinflammatory and Analgesic Activities

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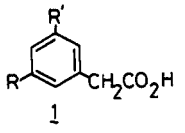
Received August 29, 1980

The positional isomers **3a–i** of 4′-chloro-5-methoxy-3-biphenylylacetic acid [1 (DKA-9), R = 4-ClPh; R′ = MeO] which is a newly developed nonsteroidal antiinflammatory agent, have been prepared and evaluated for antiinflammatory and analgesic activities using both the carrageenan-induced rat paw edema and AcOH writhing assays. The 3- and 4-biphenylylacetic acids **3a,d**, which closely resemble 1 (R = 4-ClPh, R′ = MeO) structurally, showed, by far, excellent activities compared with the other isomers in these assays. However, none of the compounds tested was more active than 1 (R = 4-ClPh; R′ = MeO). In this series of compounds, structural requirements for good antiinflammatory activity seemed to be parallel to those for analgesic activity.

Previously, we prepared a series of 3-biphenylylacetic acids (1)[1,2] and found that 4′-chloro-5-methoxy-3-biphenylylacetic acid [1 (DKA-9), R = 4-ClPh; R′ = MeO] showed excellent antiinflammatory and analgesic activities[2,3] with less tendency to cause irritation in the gastrointestinal tract than ibuprofen, indomethacin, and flufenamic acid. At that time we were interested in the effect on potency caused by structural variations. The present paper describes the synthesis of nine positional isomers (**3a–i**) of 1 (R = 4-ClPh; R′ = MeO), which are obtainable by moving the methoxy and 4-chlorophenyl groups on the benzene ring of the phenylacetic acid moiety of 1 (R = 4-ClPh; R′ = MeO), and their biological activities.



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Chemistry. Synthetic routes to the acetic acids **3a–i** are illustrated in Schemes I–VI (see paragraph at the end of this paper concerning supplementary material). The routes shown in Schemes I–IV involve the preparation of the cyclohexenones **8a–f**, **9**, and **11** (Table I) and the

(1) For the second paper of this series, see Tamura, Y.; Yoshimoto, Y.; Kunimoto, K.; Tada, S.-I.; Matsumura, S.; Murayama, M.; Shibata, Y.; Enomoto, H. *J. Med. Chem.* **1981**, *24*, 43.
(2) Tamura, Y.; Yoshimoto, Y.; Kunimoto, K.; Tada, S.-I.; Tomita, T.; Wada, T.; Seto, E.; Murayama, M.; Shibata, Y.; Nomura, A.; Ohata, K. *J. Med. Chem.* **1977**, *20*, 709.
(3) Shibata, Y. *Arzneim.-Forsch.* **1977**, *27*, 2299.

0022-2623/81/1824-1006$01.25/0