and the filtrate was acidified. The isomeric acids were separated, washed with water, and dried (8.5 g, 51%), mp 215–230°.

Separation of 6,x-Dichloro Acid. The above crude mixture was refluxed with 100 ml of ethanol and refrigerated. Almost pure title acid separated. It was recrystallized (twice) from boiling dioxane to give 3.6 g (41%) of 6,x-acid, mp 302–306° dec. Anal. ($C_{16}H_{10}Cl_2O_2S$) C, H.

Separation of the 6,y-Dichloro Acid. The ethanolic mother liquor, after separation of the previous isomeric acid, was evaporated to dryness. The residue was recrystallized from a mixture of ethanol–benzene (three times) to give 2.6 g (32%) of the 6,y-acid, mp 240–242° dec. Anal. ($C_{16}H_{10}Cl_2O_2S \cdot H_2O$) C, H.

Acknowledgment. This work was supported by the U.S. Army Medical Research and Development Command under Contract No. DADA17-69-C-9065. This is Contribution No. 1391 from the Army Research Program on Malaria. The advice and timely suggestions of Drs. T. R. Sweeney and R. E. Strube of the Walter Reed Army Institute of Research are gratefully acknowledged.

References and Notes

(1) G. R. Coatney, W. C. Cooper, N. B. Eddy, and J. Greenberg, "Survey of Antimalarial Agents", Public Health Monograph No. 9, Washington, D.C., 1953.
(2) E. L. May and E. Mosettig, *J. Org. Chem.*, 11, 629 (1946).
(3) P. L. Chien, D. J. McCaustland, W. H. Burton, and C. C. Cheng, *J. Med. Chem.*, **15**, 28 (1972).
(4) T. S. Osdene, P. B. Russell, and L. Rane, *J. Med. Chem.*, **10**, 431 (1967).
(5) (a) E. A. Nodiff, K. Tanabe, C. Seyfried, S. Matsuura, Y. Kondo, E. H. Chen, and M. P. Tyagi, *J. Med. Chem.*, **14**, 921 (1971); (b) E. A. Nodiff, A. J. Saggiomo, M. Shinbo, E. H. Chen, H. Otomasu, Y. Kondo, T. Kikuchi, B. L. Verma, S. Matsuura, K. Tanabe, M. P. Tyagi, and S. Morosawa, *ibid.*, **15**, 775 (1972); (c) E. A. Nodiff, A. J. Saggiomo, K. Tanabe, E. H. Chen, M. Shinbo, M. P. Tyagi, A. Kozuka, H. Otomasu, B. L. Verma, and D. Goff, *ibid.*, **18**, 1011 (1975).
(6) M. Pailer, A. Schleppnik, and A. Meller, *Monatsh. Chem.*, **89**, 211 (1958).
(7) D. F. DeTar, *Org. React.*, **9**, 409 (1957).
(8) P. H. Leake, *Chem. Rev.*, **56**, 27 (1956).
(9) R. E. Lutz, P. S. Bailey, M. T. Clark, J. F Codington, A. J. Deinet, E. Schwenk, F. Villiani, and E. Klingsberg, *J. Am. Chem. Soc.*, **70**, 3356 (1948).
(10) The antimalarial tests were performed by Dr. Leo Rane of the University of Miami [T. S. Osdene, P. B. Russell, and L. Rane, *J. Med. Chem.*, **10**, 431 (1967)]. See footnote *a*, Table V. Testing results were supplied through the courtesy of Drs. Thomas R. Sweeney and Richard E. Strube of the Walter Reed Army Institute of Research.

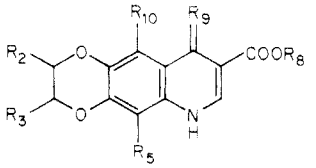
Communications to the Editor

A New Class of Diuretics with the 1,4-Dioxino[2,3-*g*]quinolone Structure

Sir:

We have synthesized a number of new 2,3,6,9-tetrahydro-9-oxo-1,4-dioxino[2,3-*g*]quinoline-8-carboxylic acid derivatives. Surprisingly, some of these compounds proved to possess an extremely high diuretic activity. The synthesis of the compounds started with 2-(hydroxymethyl)-7-nitrobenzo-1,4-dioxane,[1] which was successively alkylated with diethyl sulfate, reduced with hydrogen (Pd/C) to the amine, condensed with diethyl ethoxymethylenemalonate, and cyclized to the tricyclic ester **1**. The isomeric compound **11** and the 10-substituted derivatives **2**, **3**, and **4** were obtained similarly. Reaction of

Table I. Oral Diuretic Activity in Rats[a] and Physical Properties



R_2, R_5 = H; R_3 = $EtOCH_2$; R_8 = Et; R_9 = O[b]

No.	R_{10}	Deviations R_2–R_9	ED_{200},[c] mg/kg po	Formula	Analyses[d]	Mp,[e] °C
1	H		3.8	$C_{17}H_{19}NO_5$	C, H, N	266–268
2	Cl		0.08	$C_{17}H_{18}ClNO_5$	C, H, N, Cl	293–295
3	Br		~0.4	$C_{17}H_{18}BrNO_5$	C, H, N	258–263
4	CF_3		~0.5	$C_{18}H_{18}F_3NO_5$	H, N, F; C[f]	250–251
5	NO_2		~3	$C_{17}H_{18}N_2O_7$	H; C, N[g]	301–303
6	H	R_5 = Cl	na[h]	$C_{17}H_{18}ClNO_5$	H, Cl; C[i]	196.5–198
7	Cl	R_5 = Cl	na[h]	$C_{17}H_{17}Cl_2NO_5$	H, N; C[j]	212–214
8	Cl	R_8 = H	0.14	$C_{15}H_{14}ClNO_5$	C, H, N, Cl	266–267
9	H	R_9 = S	13	$C_{17}H_{19}NO_5S$	H, N, S; C[k]	199–202[l]
10	Cl	R_9 = NH	2	$C_{17}H_{19}ClN_2O_5$	C, H, N, Cl	174–176[m]
11	Cl	R_2 = $EtOCH_2$; R_3 = H	2.5	$C_{17}H_{18}ClNO_5$	H, N; C[n]	250–260
12	Furosemide[o]		28			
13	Chlorothiazide[p]		30			

[a] Male albino rats (strain Wistar-TNO; 160 ± 20 g). [b] Unless otherwise indicated (in the column "deviations R_2–R_9"). [c] The dose inducing a 100% increase of the urinary volume over the control value, measured over a 5-h period. [d] Unless otherwise indicated, the analyses were within ±0.4% of the theoretical values. The NMR spectra of all compounds were in agreement with the assigned structures. Most compounds were recrystallized from DMF. [e] Uncorrected; with decomposition. [f] C: calcd, 53.87; found, 53.31. [g] C: calcd, 53.96; found, 53.47. N: calcd, 7.41; found, 6.96. [h] Inactive at 50 mg/kg. [i] C: calcd, 55.51; found, 55.97. [j] C: calcd, 50.76; found, 49.93. [k] C: calcd, 58.43; found, 57.85. [l] From *i*-PrOH. [m] Hydrochloride salt (from Me_2CO–hexane). [n] C: calcd, 55.51; found, 53.42. [o] 4-Chloro-*N*-furfuryl-5-sulfamoylanthranilic acid. [p] 6-Chloro-2*H*-1,2,4-benzothiazine-7-sulfonamide 1,1-dioxide.

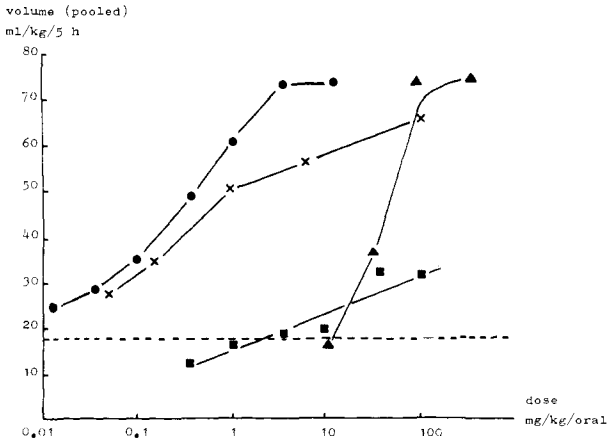


Figure 1. Dose-response relations in male albino rats (strain Wistar-TNO, 160 ± 20 g), six animals per dose group: (●-●) quincarbate, (×-×) compound **8**, (▲-▲) furosemide, (■-■) chlorothiazide, (- - -) control level.

2-chloro- and of 2-bromo-6-benzyloxy-4-nitrophenol[2] with epichlorohydrin, followed by acid debenzylation and cyclization under basic conditions, resulted in the appropriately substituted starting benzodioxanes for **2** and **3**, whereas the 2-ethoxymethyl-5-trifluoromethyl-7-nitrobenzo-1,4-dioxane was obtained by reaction of the corresponding 5-bromo compound with CF_3I.[3]

Reaction of **1** with $POCl_3$ resulted in the 9-chloro compound, which by treatment with NaSH was converted to **9**; nitration and additional acid hydrolysis of the 9-chloro compound gave **5**. Reaction of the 9-chloro derivatives of **2** with NH_3 resulted in **10**. The 5-chloro compounds **6** and **7** were obtained by direct chlorination of **1**.

The diuretic activity was estimated in saline-loaded rats after oral administration of the compounds according to a modification of the method of Lipschitz.[4] The excreted volume of urine as well as the electrolyte contents was measured over a 5-h period. The activity was expressed as an ED_{200} value, the dose which is giving a 100% increase in the urinary volume over the control value. Furosemide and chlorothiazide were used as reference compounds. An SAR study showed that the activity was limited to a rather small group of compounds. Representatives are included in Table I. Substitution with chlorine at position 5 of the tricyclic compound resulted in a strong decrease of activity (**6** and **7**). The substituents at positions 3 and 9 could be modified in a limited way without serious loss of activity (**9**, **10**, and **11**). Substitution at position 10 with lipophilic electronegative substituents, however, raised the activity considerably (**2**, **3**, and **4**). Compounds **2** and **8**, with ED_{200} values 200 times smaller than that of furosemide, were selected for further pharmacological evaluation. In the rat a steady increase of response with increasing doses was found and the designated compounds were classifiable as "high ceiling" diuretics (Figure 1). The onset of action was rapid; the duration of action was about 8 h. The compounds had a very potent natriuretic activity and Na/K ratios were comparable to those obtained with furosemide. Uric acid excretion was affected only very slightly. In rats with experimentally induced alkalosis and acidosis,[5] or edema,[6] **2** and **8** gave rise to a very effective diuretic response. After chronic administration of the compounds, no decline in diuretic response occurred. Chronic toxicity studies with doses up to 50 times the diuretic effective dose revealed no major side effects.[7] The site and mechanism of action are still under study, but inhibition of carbonic anhydrase and antagonism of aldosterone can be excluded. The diuretic activity appeared to be highly species-dependent. Activity was absent in the mouse and hamster, slight in the rhesus monkey. In nonloaded beagles the natriuretic effect was at least comparable to furosemide. Compound **2** (quincarbate)[8] is currently under clinical investigation.[9] Preliminary results indicate quincarbate to have marked diuretic and natriuretic effects in oral dosages as low as 5 mg. Further details of the chemistry and SAR will be submitted soon for publication. Reports of the pharmacological and preliminary clinical studies of quincarbate are in preparation and will be published elsewhere.

References and Notes

(1) G. B. Marini-Bettòlo and R. Landi Vittory, *Gazz. Chim. Ital.*, **87**, 1038 (1957).
(2) D. F. Page and R. O. Clinton, *J. Org. Chem.*, **27**, 218 (1962).
(3) Y. Kobayashi and I. Kumadaki, *Tetrahedron Lett.*, 4095 (1969).
(4) W. L. Lipschitz, Z. Hadidian, and A. Kerpcsar, *J. Pharmacol. Exp. Ther.*, **79**, 97 (1943).
(5) R. Z. Guzzin, J. R. Cummings, E. H. Stokey, and M. A. Ronsberg, *J. Pharmacol. Exp. Ther.*, **167**, 194 (1969).
(6) K. Meng and G. Kroneberg, *Arzneim.-Forsch.*, **17**, 659 (1967).
(7) A. Kemp and S. C. Saxena, unpublished results.
(8) International nonproprietary name.
(9) K. S. Liem, unpublished results.

J. van Dijk, J. Hartog*
Chemical Department

Th. A. C. Boschman
Pharmacological Department, Philips-Duphar Research Laboratories, Weesp, The Netherlands

Received March 5, 1976