250 ml of H_2O. A gummy precipitate was produced when the pH of the aq solution was carefully adjusted to 4. The mixture was allowed to stand for 2 hr at room temperature, the supernatant liquid was decanted, and the residual gum was triturated with abs EtOH to give 1.2 g (18%) of Ia, mp 204–207° dec. The product was recrystallized from a large volume of H_2O and dried *in vacuo* at 135° to yield analytically pure product: mp 235–237° dec; $\lambda_{max}^{pH\ 1}$ 288 mμ (ε 27,800); $\lambda_{sh}^{pH\ 1}$ 245 mμ (ε 12,400); $\lambda_{max}^{pH\ 11}$ 290 mμ (ε 29,100). *Anal.* ($C_{18}H_{20}N_8O_5$) C, H, N.

Method B.—A solution of 7.4 g (0.025 mole) of dimethyl *p*-aminobenzoyl-L-glutamate,[15] 3.0 g (0.0125 mole) of IIb and a few crystals of KI in 250 ml of abs EtOH was refluxed for 16 hr. The resulting solution was evaporated under reduced pressure and the residue was dissolved in 250 ml of H_2O. The pH of the aq solution was adjusted to 8–9 with $NaHCO_3$ which caused the precipitation of a gummy substance. No attempt was made to isolate the diester intermediate. The aq layer was decanted and to the residual gum was added 200 ml of 1 *N* NaOH. The mixture was allowed to stir at room temperature for 2 hr. The pH of the solution was carefully adjusted to 4 with 6 *N* HCl at which point the product precipitated. It was purified by recrystallization from H_2O to give 1.1 g (17%) of Ia. The product was found to be identical with that prepared by method A.

Method C.—To a solution of 3.1 g (0.007 mole) of *p*-{[(2,6-diacetamido-8-purinyl)methyl]-*N*-acetamido}benzoic acid in 250 ml of DMF cooled at 0° was added 0.7 g (0.007 mole) of Et_3N followed by 0.8 ml (0.007 mole) of ethyl chloroformate. The mixture was stirred at 0° for 1 hr. A solution of 1.5 g (0.007 mole) of dimethyl L-glutamate·HCl and 0.7 g (0.007 mole) of Et_3N in 50 ml of DMF was then added to the mixture. The resulting suspension was stirred for 20 hr at room temperature. Excess solvent was evaporated under reduced pressure at *ca.* 45°. To the residue was added 200 ml of 1 *N* NaOH. The resulting dark solution was refluxed for 30 min, purified with charcoal, and filtered. The filtrate was acidified with 6 *N* HCl to give a light yellow solid, which was recrystallized twice from H_2O to give 1 g (33%) of Ia. The product was found to be identical with that prepared by method A.

***p*-{[(2,6-Diamino-8-purinyl)methyl]-*N*-methylamino}benzoyl-L-glutamic Acid (Ib).**—A solution of 34 g (0.1 mole) of diethyl *p*-methylaminobenzoyl-L-glutamate,[16] 17 g (0.075 mole) of IIb, and a few crystals of KI in 500 ml of abs EtOH was refluxed for 48 hr. The reaction mixture was evaporated under reduced pressure and the diester intermediate was saponified by stirring the residue with 200 ml of 1 *N* NaOH at room temperature for 2 hr. The solution was then acidified to pH 4 with 6 *N* HCl and a gummy substance was formed. After decantation of the supernatant liquid, the residual gum was triturated with absolute EtOH. The resulting yellow solid was collected by filtration to give 10 g of crude product. Recrystallization from H_2O gave, after being dried at 135° *in vacuo*, 4.5 g (12%) of analytically pure Ib: mp 216–218°; $\lambda_{max}^{pH\ 1}$ 292 mμ (ε 29,200); $\lambda_{sh}^{pH\ 1}$ 249 mμ (ε 13,200); $\lambda_{max}^{pH\ 11}$ 295 mμ (ε 32,800). *Anal.* ($C_{19}H_{22}N_8O_5$) C, H, N.

Acknowledgment.—The authors wish to thank Mrs. Margaret L. Rounds and Mr. John R. Gravatt for the analytical and instrumental measurements.

(15) R. Koehler, L. Goodman, J. DeGraw, and B. R. Baker, *J. Amer. Chem. Soc.*, **80**, 5779 (1958).

(16) S.-C. J. Fu and M. Reiner, *J. Org. Chem.*, **30**, 1277 (1965).

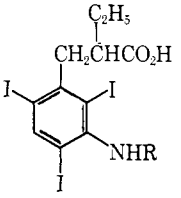
Sodium Tyropanoate,[1] a New Oral Cholecystographic Agent

J. O. HOPPE,[2a] J. H. ACKERMAN,[2b] A. A. LARSEN, AND J. MOSS

Sterling-Winthrop Research Institute, Rensselaer, New York 12144

Received May 11, 1970

Cholecystography is the roentgenographic visualization of the gallbladder after an administered radiopaque substance or its metabolite has been secreted in the bile and collected in the gallbladder. We wish to report that sodium 3-butyramido-α-ethyl-2,4,6-triiodohydrocinnamate, sodium tyropanoate[1] (**5**), showed favorable characteristics in laboratory studies as an oral cholecystographic agent.



	R
1	H
2	COH
3	$COCH_3$
4	COC_2H_5
5	$CO(CH_2)_2CH_3$ (Na salt)
6	$CO(CH_2)_3CH_3$
7	$CO(CH_2)_4CH_3$

Sodium tyropanoate (**5**) is a derivative of iopanoic acid (**1**) and was found to excel iopanoic acid and other oral agents[3–10] in one or more laboratory studies. Table I compares the acute toxicities in mice and the average cholecystographic indexes[11] (ACI) in cats of Na tyropanoate and other agents. Both iv and oral toxicities are given in Table I although there are shortcomings in the comparison of compounds by each method. Since only oral administration is used in the clinic for these agents, there is not necessarily a direct relationship between the acute iv toxicity and the adverse effects observed in the clinic. The acute oral toxicities may be ineffective for comparison purposes because of the difficulty of giving a massive dose which is required to produce mortality in animals in a manner which corresponds to the clinical administration. The method of Hoppe and Archer[11] was used for determining the acute oral toxicities reported in Table I. This consisted of administering the materials as a powder suspended in H_2O with gum tragacanth in a volume of 0.5 ± 0.35 ml by stomach tube. In the clinic iopanoic acid is administered in tablets and Na tyropanoate in capsules. Large variations in the acute oral toxicity can exist from sample to sample for some agents. For iopanoic acid the toxicities varied from 6.6 to 15.8 g/kg (24 hr) and 5.9 to 13.9 g/kg (7 days) for a series of more than 10 samples. Most 7-day values were between 6 and 10 g. The reason for this broad range was not readily determined. It is speculated that the cause is a combination of differences in particle size and crystalline habit. Other workers reported acute oral LD_{50} values of 5.12[12] and 3.87[13] g/kg in mice for iopanoic acid but did not describe their methods.

With samples of Na tyropanoate (**5**) values in the acute oral LD_{50} toxicities ranged from 4.8 to 16.3 g/kg. This variation is believed to be due to different methods of crystallization which produced crystals with different

(1) Bilopaque.

(2) (a) Deceased; (b) author to whom inquiries should be addressed.

(3) E. A. Graham and W. H. Cole, *J. Amer. Med. Ass.*, **82**, 613 (1924).

(4) M. Dohrn and P. Diedrick, *Deut. Med. Wochenschr.*, **66**, 1133 (1940); N. Kleiber, *ibid.*, **66**, 1134 (1940); K. Junkmann, *Klin. Wochenschr.*, **20**, 125 (1941).

(5) T. R. Lewis and S. Archer, *J. Amer. Chem. Soc.*, **71**, 3753 (1949).

(6) D. Papa, H. F. Ginsberg, I. Lederman, and V. DeCamp, *ibid.*, **75**, 1107 (1953).

(7) M. Chao and P-C. Hu, *Hua Hsüeh Hsüeh Pao*, **23**, 361 (1957); *Chem. Abstr.*, **52**, 15467*g* (1958).

(8) H. Priewe and A. Poljak, *Chem. Ber.*, **93**, 2347 (1960).

(9) British Patent 870,321; *Chem. Abstr.*, **55**, 24683*f* (1961).

(10) J. A. Korver, *Recl. Trav. Chim. Pay-Bas*, **87**, 308 (1968).

(11) J. O. Hoppe and S. Archer, *Amer. J. Roentgenol. Radium Ther Nucl. Med.*, **69**, 630 (1953).

(12) R. Shapiro, *Radiology*, **60**, 687 (1953).

(13) K. Winzer, H. Langecker, and K. Junkmann, *Aertzl. Wochenschr.*, **9**, 950 (1954).

TABLE I

ACUTE TOXICITIES IN THE MOUSE AND AVERAGE CHOLECYSTOGRAPHIC INDEXES (ACI) IN THE CAT FOR SOME ORAL CHOLECYSTOGRAPHIC AGENTS

Compound[a]	Acute intravenous LD_{50}[b] mouse,[c] expressed as mg/kg	expressed as mg-atoms of iodine per kg	Oral LD_{50}[d] suspension, g/kg of mouse[d]	ACI, 100 mg/kg cat[e]	Ref
Iodophthalein sodium	360 ± 12	1.53 ± 0.05			3
Iodoalphionic acid	400 ± 20[f]	1.62 ± 0.08	3.8 ± 0.3[f]	2.5[f]	4
Iopanoic acid	321 ± 20[f]	1.68 ± 0.11	6.6 ± 0.7 to 15.8 ± 1.1 (24 hr)[g]	3.6[f]	5
Na tyropanoate	720 ± 56	3.26 ± 0.25	16.3 ± 1.83 to 4.8 ± 1.45	3.7	
Iophenoxic acid	374 ± 30	1.96 ± 0.16		3.0	6
Buniamiodyl	418 ± 23 570[h]	1.90 ± 0.10 2.59	2.78 ± 0.64 (24 hr) and 2.89 ± 0.58 (7 day)	3.7	7
Na ipodate	290 ± 26 300[i]	1.40 ± 0.12 1.45	2.57 ± 0.48	3.6	8
Iobenzamic acid	530 ± 42 480[i]	2.41 ± 0.19 2.18		3.1	9
Iocetamic acid	410 ± 22	2.01 ± 0.11		3.0	10

[a] The structures of these compounds are all given in Chapter 67 of "Medicinal Chemistry," A. Burger, Ed., Wiley, New York, N. Y., 1970, and also in "The Merck Index," 8th ed, except for iocetamic acid [*N*-acetyl-*N*-(3-amino-2,4,6-triiodophenyl)-2-methyl-β-alanine]. [b] ± Standard error. [c] The method described by J. O. Hoppe, A. A. Larsen, and F. Coulston, *J. Pharmacol. Exp. Ther.*, **116**, 394 (1956), was followed for determining the acute iv toxicities. [d] The method described in ref 11 was followed for the determination of the acute oral toxicities. [e] The method described in ref 11 was followed for the determination of the average cholecystographic indexes (ACI). The scoring scheme may be briefly described as 0 = no visualization; 1 = faint evidence of gallbladder concentration; 2 = faint shadow of gallbladder; 3 = distinct shadow of gallbladder; 4 = sharp outline of the gallbladder. [f] Reference 11. [g] The 7-day values were 5.9 ± 2.5 to 13.9 ± 1.0, most were between 6 and 10 g for a series of more than 10 samples. [h] I. Levenstein, A. Wolven, and A. Urdang, *J. Pharm. Soc.*, **50**, 959 (1961). [i] K. H. Kimbel and H. Langecker, *Acta Radiol.*, **55**, 305 (1961). [j] I. Lindner, H. Stormann, W. Obendorf, and R. Kilches, *Arzneimittl. Forsch.*, 11, 384 (1961).

rates of solution. The inherent difficulties of determining acute oral toxicities by the administration of solids could be avoided by the use of aqueous solutions, but we believe this would correspond even less to clinical procedure than the method used here. In addition, an agent like iopanoic acid would have to be converted first to a water-soluble salt. Despite these problems, the acute oral and intravenous toxicities are the usual manner for obtaining a preliminary assessment of the safety of oral cholecystographic agents.

Sodium tyropanoate (**5**) is the Na salt of the butyryl derivative of iopanoic acid (**1**). Other acyl derivatives of **1** which contain 1 to 6 C atoms in the acyl group were made and Table II shows the acute iv LD_{50} values in mice and the ACI values in cats of this series of compounds. All of these compounds showed good to excellent gallbladder visualization in cats except for the formyl derivative, and the toxicities increased with increasing size of the acyl group excluding the formyl derivative. 3-Acetamido-α-ethyl-2,4,6-triiodohydrocinnamic acid[14] (*N*-acetyliopanic acid, **3**), is the least toxic member of this series and was previously investigated in the clinic.[15] It had a marked reduction in observed side effects in comparison to iopanoic acid, but unfortunately, there was a decrease in the efficiency of gallbladder visualization and it did not appear to have any advantage. The poor visualization found in the clinic with **3** is attributed to too much loss of material by urinary excretion. Na tyropanoate, which has 2 more methylenes on the acyl group than *N*-acetyliopanoic acid, was prepared with the expectation that it would have less urinary and more biliary excretion than

TABLE II

ACYL DERIVATIVES OF IOPANOIC ACID

Acyl Group	Formula	Acute intravenous LD_{50}[a,b] expressed as mg/kg	expressed as mg-atoms of iodine per kg	Oral ACI, 100 mg/kg[c]
Formyl	2	790 ± 54 (24 hr) 760 ± 61 (7 day)	3.95 ± 0.27 3.80 ± 0.31	1.11
Acetyl	3	1020 ± 57	4.82 ± 0.27	3.5
Propionyl	4	820 ± 44	3.79 ± 0.20	3.6
Butyryl	5	720 ± 56	3.26 ± 0.25	3.7
Valeryl	6	510 ± 26	2.22 ± 0.11	3.8
Hexanoyl	7	335 ± 29	1.52 ± 0.13	3.3

[a] ± Standard error. [b] Na salt solns were used in these toxicity studies. [c] Na salts were used for all ACI studies except for **2** and **3**.

N-acetyliopanoic acid. This followed from the studies of Epstein[16] and coworkers and Hoppe and Archer[14] which showed that the addition of CH_2 groups to a potential cholecystographic agent may increase the biliary excretion. Clinical results indicated that this supposition was correct since Na tyropanoate produced gallbladder visualization equivalent to or better than iopanoic acid[17–22] while the *N*-acetyliopanoic (**3**) acid gave visualization inferior to that of iopanoic acid.[15,17] The Na salt of tyropanoic acid was preferred because animal studies showed more consistent visualization of

(14) S. Archer and J. O. Hoppe, U. S. Patent 2,895,988 (1959); *Chem. Abstr.*, **54**, 1446a (1960).

(15) W. M. Whitehouse and A. S. Shufro, *Univ. Mich. Med. Bull.*, **20**, 231 (1954).

(16) B. S. Epstein, S. Natelson, and B. Kramer, *Amer. J. Roentgenol. Radium Ther. Nucl. Med.*, **56**, 201 (1946).

(17) In the clinical comparisons of Na tyropanoate (**5**) and iopanoic acid (**1**), the doses of **5** were 3.0 and 4.5 g while the doses of **1** were 3.0 g. In the comparison of **3** and **1**, each was given in a 2.0-g dose.

(18) G. J. Benisek and J. A. Gunn, *Amer. J. Roentgenol. Radium Ther. Nucl. Med.*, **88**, 792 (1962).

(19) S. Pollack, *J. Okla. State Med. Ass.*, **56**, 13 (1963).

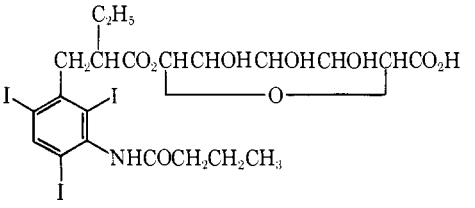
(20) R. J. Reeves, *Southern Med. J.*, **56**, 351 (1963).

(21) H. J. Borhenne, *Radiology*, **81**, 629 (1963).

(22) D. J. Hurley and D. R. Feore, *J. Can. Ass. Radiol.*, **14**, 187 (1963).

the gallbladder with the salt than with the free acid. The side effects observed with Na tyropanoate in the clinic were less than those seen with iopanoic acid.[18,20-22]

A crossover study in man by McChesney and Banks showed that 50% of a 4.5-g dose of Na tyropanoate is excreted in the urine in 108 hr while 37% of a 3-g dose of iopanoic acid was in the urine.[23] McChesney and Hoppe reported that iopanoic acid and Na tyropanoate are metabolized in the cat and man and that the biliary excretion is mostly as the glucuronic acid conjugates.[24-27] The suggested metabolite of Na tyropanoate is shown in 8.



8

Experimental Section[28]

α-Ethyl-3-formamido-2,4,6-triiodohydrocinnamic Acid (2), Na 3-Acetamido-α-ethyl-2,4,6-triiodohydrocinnamate (3, Na Salt), and Na α-Ethyl-2,4,6-triiodo-3-propionamidohydrocinnamate (4, Na Salt).—The preparation of the first two of these compounds by the acylation of iopanoic acid[5] is described elsewhere.[14] The acid **4** corresponding to the last of the above compounds is also described.[14] The Na salt of **4** was prepared by the method employed for Na tyropanoate and was obtained as colorless crystals, mp 199–210°. *Anal.* ($C_{14}H_{15}I_3NNaO_3$) C, H; I: calcd, 58.66; found, 58.06.

Tyropanoic Acid, 3-Butyramido-α-ethyl-2,4,6-triiodohydrocinnamic Acid (5, Acid).—A mixture of 50.0 g (0.0875 mole) of iopanoic acid[5] (1), 28.6 ml (0.175 mole) of butyric anhydride, 310 ml of $PrCO_2H$, and 5 drops of H_2SO_4 was heated on a water bath at 70–80° for 2 hr. A solution formed and was poured into H_2O. The solid which separated was collected and dried, 47.0 g (84%) of tan solid, neutralization equiv, 637; calcd for $C_{15}H_{18}I_3NO_3$: neutralization equiv, 641. Recrystallization from EtOAc gave very pale tan prisms, mp 182–184° (reported mp 172–185.5°[14]); neutralization equiv 640; uv max (95% EtOH) 237 mμ (ε 33,900); ir (3/4% KBr disc) 1660 (CONH), 1690 (COOH), 2500–2670 (broad H bonding), 2940 (CH), and 3220 cm^{-1} (NH).

Na Tyropanoate, [Na 3-Butyramido-α-ethyl-2,4,6-triiodohydrocinnamate (5)].—Tyropanoic acid (**5**, acid) was converted into its Na salt by the addition of a slight excess of methanolic NaOH to a suspension of **5** (acid) in MeOH. A solution was obtained and a gummy material separated when Et_2O was added. The addition of fresh Et_2O to the residue after the liquid layer was decanted and trituration produced a solid which was collected and dried. There was obtained a colorless solid, mp 208–210°. *Anal.* ($C_{15}H_{17}I_3NNaO_3$) C, H; I: calcd, 57.42; found, 56.6. Other samples of Na tyropanoate were recrystallized from H_2O and aq *i*-PrOH.

Na α-Ethyl-2,4,6-triiodo-3-valeramidohydrocinnamate (6, Na Salt).—The reaction of iopanoic acid[5] with valeric anhydride in the presence of valeric acid and H_2SO_4 in the manner described for tyropanoic acid (**5**, acid) gave α-ethyl-2,4,6-triiodo-3-valeramidohydrocinnamic acid (**6**). Recrystallization (EtOH) gave colorless prisms, mp 189–190.5°. *Anal.* ($C_{16}H_{20}I_3NO_3$) neutralization equiv: calcd., 655; found 652. The Na salt of **6** was obtained as colorless solid, mp 212–217° dec, from **6** in the manner described for Na tyropanoate. *Anal.* ($C_{16}H_{19}I_3NNaO_3$)C, H; I: calcd, 56.23, found, 56.75.

Na α-Ethyl-3-hexanamido-2,4,6-triiodohydrocinnamate (7).—The reaction of iopanoic acid[5] with hexanoyl anhydride and H_2SO_4 gave α-ethyl-3-hexanamido-2,4,6-triiodohydrocinnamic acid (**7**) as colorless prisms (EtOH), mp 196–198°. *Anal.* ($C_{17}H_{22}I_3NO_3$) C, H; I: calcd, 56.90; found, 56.01. The Na salt of **7** was prepared from the acid in the manner described for Na tyropanoate (**5**) and was obtained as a colorless solid, mp 170–190°. *Anal.* ($C_{17}H_{21}I_3NNaO_3$) C, H, **I**.

Acknowledgments.—Appreciation is expressed to Mr. John Romano, Mr. Chester Sapino, and Mr. Arnold Ludke for technical assistance. The authors wish to thank the Analytical and Physical Chemistry Departments at Sterling-Winthrop Research Institute for the analytical and spectral data. We also wish to thank Dr. H. P. Drobeck and Mr. L. Duprey for the acute toxicity studies.

(23) E. W. McChesney and W. F. Banks, Jr., *Proc. Soc. Exp. Biol. Med.*, **119**, 1027 11965).

(24) E. W. McChesney and J. O. Hoppe, *Arch. Intn. Pharmacodyn.*, **99**, 127 (1954).

(25) E. W. McChesney and J. O. Hoppe, *ibid.*, **105**, 306 (1956).

(26) E. W. McChesney and J. O. Hoppe, *ibid.*, **142**, 562 (1963).

(27) E. W. McChesney, *Biochem. Pharmacol.*, **13**, 1366 (1964).

(28) When analyses are indicated only by symbols of elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. Melting points were taken in a Hershberg-type apparatus and are corrected. The spectra were determined on a Cary 15 ultraviolet spectrophotometer and on a Perkin-Elmer 21 infrared spectrophotometer.

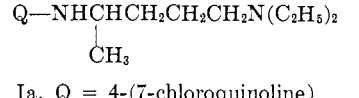
Isoquinolines. 2. 3-(Dialkylaminoalkylamino)isoquinolines as Potential Antimalarial Drugs[1,2]

JOHN L. NEUMEYER[3] AND KLAUS K. WEINHARDT

Arthur D. Little, Inc., Acorn Park, Cambridge, Massachusetts 02140

Received March 13, 1970

Because quinolines have played such an important role in malaria chemotherapy, we believed that the heretofore unexplored class of 3-aminoisoquinolines deserved further investigation. In our previous report[1] we presented the synthesis and biological activity of a number of 3-aminoisoquinolines which do not contain the usual dialkylaminoalkylamino side chain, a common feature of the active quinoline antimalarials such as chloroquine (Ia) or pamaquine (Ib).



Ia, Q = 4-(7-chloroquinoline)
Ib, Q = 8-(6-methoxyquinoline)

This report will present the synthesis and biological activity of such isoquinoline derivatives.

Chemistry.—The synthesis of the diamines (VI) was carried out by the sequence of reactions shown in Scheme I from the appropriately substituted aminoisoquinoline[1] (II). The attempted alkylation of the 3-chloropropionamide **27** with *N*-methylaniline yielded only the elimination product, *N*-(3-isoquinolyl)acrylamide.[4a] Such an elimination also occurred when the

(1) Paper **1**: J. L. Neumeyer and K. K. Weinhardt, *J. Med. Chem.*, **13**, 613 (1970).

(2) This work was supported by the U. S. Army Medical Research and Development Command under Contract DA-49-193-MD-3023. This is Contribution No. 783 from the Army Research Program on Malaria. Presented in part at the 155th National Meeting of the American Chemical Society, Miami, Florida, 1968, N-28.

(3) To whom inquiries should be addressed at the Department of Medicinal Chemistry, College of Pharmacy, Northeastern University, Boston, Mass. 02115.

(4) (a) This compound was described in the previous paper (ref 1) and was designated as compound **14**; (b) This compound was described in the previous paper (ref 1) and was designated as compound **15**.