TABLE II

1-AROYL-3-THIOSEMICARBAZIDES

RCONHNHCSNH$_2$

R	Method of prepn.	Recrystn. solvent	Yield, %	M.p., °C.	Formula	Calcd., % C	Calcd., % H	Calcd., % N	Found, % C	Found, % H	Found, % N
p-$CH_3CO_2C_6H_4$	O	*a*	23	210–211	$C_{10}H_{11}N_3O_3S$	45.47	4.30	15.18	45.77	4.54	15.06
p-$H_2NSO_2C_6H_4$	P	*b*	95	231–233 dec.	$C_8H_8O_2S_2$	...	...	20.43	...	...	20.34
p-$C_2H_5SO_2C_6H_4$	O	*b*	80	219–220	$C_{10}H_{13}N_3O_3S_2$	41.79	4.57	...	41.98	4.60	...
4,2-$H_2N(HO)C_6H_3$	P	*b*	24	210–212	$C_8H_{10}N_4O_2S$	...	...	24.76	...	...	24.90
2-Pyridyl	P	*b*	86	197–199 dec.	$C_7H_8N_4OS$	...	...	28.55	...	...	28.21
4-Quinolyl	P	*b*	80	184–186 dec.	$C_{11}H_{10}N_4OS$	53.64	4.10	...	53.34	4.48	...
2-Pyrazinyl	P	*b*	95	222–223 dec.	$C_6H_7N_5OS$	36.54	3.58	35.53	36.74	3.70	35.45

[a] 95% ethanol. [b] Water.

at 250–252° dec. (*Anal.* Calcd. for $C_{11}H_{11}N_5O_2$: N, 28.57. Found: N, 28.19.). The crude product from the previous step (9.0 g.), 200 ml. of 95% ethanol, and 2.5 ml. of 85% hydrazine hydrate were refluxed for 4 hr. and cooled, and the solid was filtered. The air-dried material, 5.6 g., was recrystallized from aqueous N,N-dimethylformamide to give 4.5 g. of **33**.[3]

4-Amino-3,5-bis(2-furyl)-*s*-triazole (38). Method M.—The reaction between 33.0 g. (0.15 mole) of 1,2-bis(2-furoyl)hydrazine and 15.0 g. (0.40 mole) of 85% hydrazine hydrate by the literature procedure[4] gave 5.7 g. of **38**.

N-(3,5-Di-4-pyridyl-*s*-triazol-4-yl)isonicotinamide Dihydrochloride (40). Method N.—To 20.0 g. (0.084 mole) of **39** in 100 ml. of pyridine, at 0–5°, was added in portions 17.8 g. (0.1 mole) of sublimed isonicotinyl chloride hydrochloride. The reaction mixture was stirred for 18 hr. at room temperature, heated for 3 hr. on the steam bath, cooled, and treated with 250 ml. of ice water. The precipitated solid was filtered and dried to give 8.0 g. of **39**. To the filtrate was added 20 ml. of concentrated aqueous NH_3 and the solution was concentrated to dryness *in vacuo*. The residue was dissolved in 600 ml. of boiling absolute ethanol and allowed to cool to room temperature, the NH_4Cl was filtered, the filtrate was concentrated to 200 ml. and again filtered, and the filtrate was diluted with 400 ml. of hexane. The solid which separated was filtered and dried to give 9.3 g. (32% yield) of crude base, m.p. 267–268° dec., but this compound could not be purified by recrystallization.

To the base, 6.9 g. (0.02 mole) in 150 ml. of absolute ethanol, was added 0.062 mole of HCl in ether solution. The crystalline product was filtered and recrystallized from 90% methanol to give 6.2 g. of **40**.

1-(*p*-Acetoxybenzoyl)-3-thiosemicarbazide. Method O.—To 2.30 g. (0.25 mole) of powdered semicarbazide and 40 ml. of pyridine, with ice-water cooling, was added dropwise 49.6 g. (0.25 mole) of *p*-acetoxybenzoyl chloride in 50 ml. of dry benzene. The mixture was stirred for 4 hr. at room temperature and diluted with 200 ml. of water, and the oily solid was filtered. Recrystallization from 95% ethanol gave 14.5 g. of product.

1-(*p*-Sulfamoylbenzoyl)-3-thiosemicarbazide. Method P.—A mixture of 21.5 g. (0.1 mole) of *p*-sulfamoylbenzoyl hydrazide, 7.1 g. (0.1 mole) of dry ammonium thiocyanate, and 8.6 g. of concentrated HCl in 90 ml. of water was heated on the steam bath for 16 hr. and then cooled; the solid was filtered and air dried to give 26 g. of product.

(3) D. W. Kaiser and G. A. Peters [*J. Org. Chem.*, **18**, 196 (1953)] have described the formation of 3-ureido-5-aryl-*s*-triazoles by this reaction; they did not prepare **33**.

(4) R. M. Herbst and J. A. Garrison, *ibid.*, **18**, 872 (1953). It is of interest that A. Pinner [*Ann.*, **298**, 32 (1897)] obtained **38**, m.p. 245°, by heating 3,6-bis(2-furyl)-1,2-dihydro-1,2,4,5-tetrazine in 25% HCl but reported the product to be 3,6-bis(2-furyl)-1,4-dihydro-1,2,4,5-tetrazine. R. Stolle [*J. prakt. Chem.*, [2] **75**, 416 (1907)] showed that 3,6-disubstituted dihydrotetrazines, but not including Pinner's compound, when so treated gave triazoles. Hence, F. K. Beilstein ("Handbuch der organischen Chemie," Vol. 27, 4th ed., 1919, p. 790) lists **38** by the above corrected structure.

Iodinated 5- and 8-Hydroxyisoquinolines as Potential Amebicides

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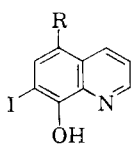
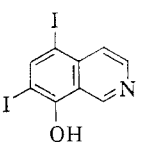
Some iodinated 5- and 8-hydroxyisoquinolines have been synthesized and evaluated for antiamebic activity *in vitro* and *in vivo* in comparison with Vioform. With the exception of 5,7-diiodo-8-isoquinolinol (III) and 5-iodo-8-isoquinolinol (VII), which were weakly active when tested *in vitro* against *Endamoeba histolytica*, none of the substances showed antiamebic activity at the doses employed.

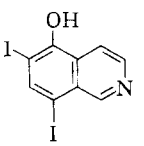
Various iodinated 8-hydroxyquinolines such as Diiodoquin (I) and Vioform (II) are frequently used in the prophylactic and therapeutic treatment of intestinal amebiasis. We wish to report the synthesis of the isomeric isoquinoline analogs III and IV of Diiodoquin and the results of the evaluation of their antiamebic properties.



I, R = I
II, R = Cl



III

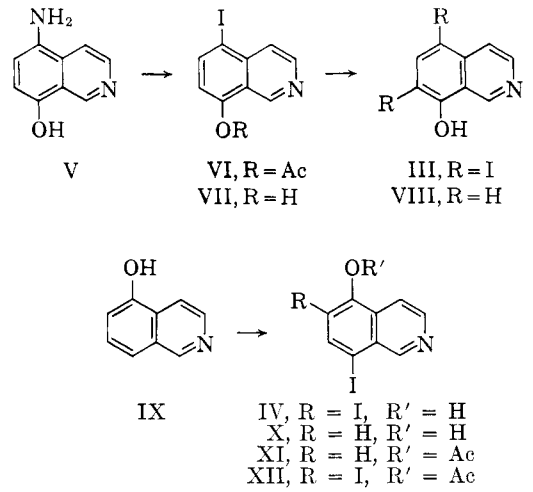


IV

8-Isoquinolinol (VIII) is of potential interest as a starting material for the synthesis of 5,7-diiodo-8-isoquinolinol (III). This compound has been described by Robinson,[1] who prepared it in an overall yield of 15% by sulfonation of isoquinoline at 300° followed by alkali fusion of the resulting sulfonic acid mixture. Since the structure of VIII had been assigned solely on the basis of nonidentity with 5-, 6-, and 7-hydroxyisoquinoline, we decided to refrain from the use of Robinson's method for the preparation of this compound and, instead, utilized the *p*-aminophenol V in the synthesis of III (Scheme I). The diazonium

(1) R. A. Robinson, *J. Am. Chem. Soc.*, **69**, 1944 (1947).

SCHEME I



salt of 5-amino-8-isoquinolinol (V)[2] was treated with potassium iodide and iodine. Treatment of the complex reaction mixture with sodium sulfite and sodium hydroxide followed by neutralization gave a precipitate which, on acetylation with acetic anhydride in pyridine, afforded the pure phenol acetate VI in an over-all yield of 57%. Alkaline hydrolysis of VI gave a quantitative yield of the monoiodophenol VII as the free base; the corresponding hydrochloride was obtained by hydrolysis of VI with hydrochloric acid. Iodination of this hydrochloride with iodine chloride in ethanol[3] finally produced the desired 5,7-diiodo-8-isoquinolinol (III) as its hydrochloride in 66% yield. It should be mentioned also that the catalytic reduction (in alkaline solution[4]) of VII gave 8-hydroxyisoquinolinol (VIII) which was identical with the isoquinolinol prepared according to Robinson.[1]

The easily accessible 5-isoquinolinol (IX)[5] was selected as the starting material for the synthesis of 6,8-diiodo-5-isoquinolinol (IV). Iodination of IX with iodine chloride in ethanol resulted in a mixture of iodinated products from which a pure monoiodo derivative was isolated in the form of its hydrochloride. Structure X was tentatively assigned to this compound which was also characterized as its free base and acetate XI. When the iodination was carried out in 3 *N* hydrochloric acid instead of ethanol, a product was obtained which, after acetylation with acetic anhydride in pyridine, afforded the pure diiodo acetate XII in 53% over-all yield. Alkaline hydrolysis of XII yielded, quantitatively, the desired 6,8-diiodo-5-isoquinolinol (IV). The structural assignments of the compounds described are supported by spectroscopic data (see Experimental Section).

All of the iodoisoquinolines prepared (except XII) were tested against *Endamoeba histolytica in vitro*. In addition, compounds III, IV, and XII were examined for their effect *in vivo* against the intracecal *E. histolytica* K-9 infection of weanling rats using the method described by Jones.[6] The results of these experiments are given in Table I.

TABLE I

In Vitro AND *in Vivo* ACTIVITY OF IODINATED 5- AND 8-HYDROXYISOQUINOLINES AGAINST *E. histolytica*

Compd.	*In vitro*[a] MIC,[b] μg./ml.	*In vivo*[c] PD_{50},[d] mg./kg. *p.o.*
III	1000	300, inactive
IV	1000, inactive	300, inactive
VI	1000, inactive	Not tested
VII	1000	Not tested
X	1000, inactive	Not tested
XI	1000, inactive	Not tested
XII	Not tested	300, inactive
II	62.5	456

[a] We are indebted to Dr. H. Scholer and his staff (F. Hoffmann-La Roche and Co., Basle, Switzerland) for carrying out this test. [b] MIC = minimal inhibiting concentration. [c] We thank Dr. E. Grunberg and his staff for the determination and the interpretation of these data. [d] PD_{50} = 50% protective dose.

Activity *in vitro* was seen with III and VII only at the maximum concentration tested (1000 μg./ml.); all other compounds were without effect. Vioform (II), used as a standard, exhibited its expected activity *in vitro*. None of the substances tested showed activity *in vivo* against *E. histolytica* at the doses employed, whereas Vioform exhibited its expected moderate antiamebic activity.

Experimental Section[7]

5-Iodo-8-isoquinolinol Acetate (VI).—A solution of 4 g. of $NaNO_2$ in 40 ml. of water was added at 5–7° to a suspension of 10.8 g. (30 mmoles) of 5-amino-8-isoquinolinol disulfate[2] in 88 ml. of 17% HCl and 14 g. of ice. The resulting solution was kept for 15 min. at 5–7°, and excess HNO_2 was decomposed by addition of 4 g. of urea in 40 ml. of water. The mixture was stirred until gas evolution ceased (*ca.* 20 min.), some insoluble material was removed by filtration, and the filtrate was added to a 70° solution of 30 g. of KI and 9 g. of iodine in 60 ml. of water. The mixture was cooled overnight in the refrigerator, and the black precipitate was filtered and treated with 70 ml. of 10% aqueous Na_2SO_3 for 15 min. To the pale brown suspension was added 120 ml. of 1 *N* NaOH. The resulting black solution was cooled and adjusted to pH 6.5 by addition of concentrated HCl. Filtration afforded 9 g. of a brown powder which was dissolved, at room temperature, in 80 ml. of acetic anhydride–pyridine (1:1). After 30 min., the reaction mixture was evaporated to dryness *in vacuo* and the residue, in CH_2Cl_2, was filtered through a column of Florisil (50 g.). The CH_2Cl_2 eluate was treated with charcoal and taken to dryness. The residue was crystallized from ether–hexane to give 5.5 g. (57%) of VI: m.p. 115°; λ_{max}, mμ (ε), 211 (33,000), 290 (sh) (5700), 300 (6250), 323 (5000), 333 (5350); ν_{max} 1780, 1770 cm.$^{-1}$; δ 9.25 (1H, s, C-1 H), 8.66 (1H, d, C-3 H), 8.25 (1H, d, C-6 H), 7.85 (1H, d, C-4 H), 7.18 (1H, d, C-7 H), 2.50 (3H, s, CH_3).

Anal. Calcd. for $C_{11}H_8INO_2$: C, 42.20; H, 2.58; I, 40.53; N, 4.47. Found: C, 42.46; H, 2.76; I, 40.55; N, 4.16.

5-Iodo-8-isoquinolinol Hydrochloride.—A solution of 9.4 g. (30 mmoles) of VI in 90 ml. of 3 *N* HCl was stirred for 2 hr. at room temperature. Upon addition of 270 ml. of concentrated HCl, a yellow crystalline product was formed. The crystals were filtered, washed with water, cold ethanol, and ether, and dried in a desiccator to give 8.3 g. (92%) of pure 5-iodo-8-isoquinolinol

(2) L. F. Fieser and E. L. Martin, [*J. Am. Chem. Soc.*, **57**, 1840 (1935)] obtained this compound by electrolytic reduction of 5-nitroisoquinoline. These authors did not know whether their starting material was 5- or 8-nitroisoquinoline. From the work of F. T. Tyson [*ibid.*, **61**, 183 (1939)], it was concluded that the compound in question was 5-nitroisoquinoline; *cf.* M. J. S. Dewar and P. M. Maitlis, *J. Chem. Soc.*, 2521 (1957).

(3) *Cf.* A. Das and S. L. Mukherji, *J. Org. Chem.*, **22**, 1111 (1957).

(4) H. Kämmerer, L. Horner, and H. Beck, *Chem. Ber.*, **91**, 1376 (1958).

(5) V. Georgian, R. J. Harrisson, and L. L. Skaletzky, *J. Org. Chem.*, **27**, 4571 (1962).

(6) W. R. Jones, *Ann. Trop. Med. Parasitol.*, **40**, 130 (1946).

(7) All melting points were determined on a Thomas–Hoover capillary melting point apparatus and are uncorrected. Ultraviolet spectra (in isopropyl alcohol unless otherwise noted) were taken on a Cary recording spectrophotometer, Model 14M. Infrared spectra (in $CHCl_3$) were either measured on a Perkin-Elmer infrared spectrophotometer, Model 337, or on a Beckman instrument, Model IR-9. N.m.r. spectra were measured in $CDCl_3$ on a Varian A-60 instrument and chemical shifts are reported in parts per million (δ) downfield from an internal tetramethylsilane reference. The abbreviations s for singlet and d for doublet are used.

hydrochloride: m.p. 210–211°; λ_{max}, mμ (ϵ), 226 (sh) (20,750), 243 (sh) (14,850), 260 (sh) (3750), 315 (4500), 341 (5200).

Anal. Calcd. for $C_9H_6INO \cdot HCl$: C, 35.15; H, 2.30; N, 4.56. Found: C, 35.47; H, 2.37; N, 4.56.

5-Iodo-8-isoquinolinol (VII).—A solution of 300 mg. (0.96 mmole) of VI in 10 ml. of methanol was added to 20 ml. of 1 *N* NaOH, and the resulting mixture was stirred for 30 min. at room temperature. The solution was then adjusted to pH 7.0 with 1 *N* HCl. After filtration, washing with water, and drying in a desiccator, 258 mg. (100%) of VII, m.p. 185–188°, was obtained: λ_{max}, mμ (ϵ), 233 (sh) (16,000), 242 (16,250), 315 (5250), 343 (6400).

Anal. Calcd. for C_9H_6INO: C, 39.88; H, 2.23; I, 46.82; N, 5.17. Found: C, 39.69; H, 2.66; I, 47.10; N, 5.12.

5,7-Diiodo-8-isoquinolinol Hydrochloride.—With stirring, 17.7 g. (109 mmoles) of iodine monochloride in 470 ml. of ethanol was added to a suspension of 8.3 g. (27 mmoles) of VII hydrochloride in 110 ml. of ethanol. The reaction mixture was stirred overnight at room temperature. The product was collected by filtration and washed with cold ethanol and ether to yield 8.1 g. (70%) of 5,7-diiodo-8-isoquinolinol hydrochloride, m.p. 208–210°. An analytical sample was prepared as follows. The hydrochloride (2 g.) was dissolved at room temperature in 30 ml. of dimethylformamide and 500 ml. of methanol. Addition of 500 ml. of ether gave 1.1 g. of yellow crystals, m.p. 209–210°, which after recrystallization from the same solvent mixture gave an analytically pure sample: m.p. 209–210°; λ_{max}, mμ (ϵ), 225 (sh) (21,000), 251 (18,850), 313 (4450), 349 (4750).

Anal. Calcd. for $C_9H_5I_2NO \cdot HCl$: N, 3.23; total halogen, 66.74. Found: N, 3.58; total halogen, 66.95.

6,8-Diiodo-5-isoquinolinol Acetate (XII).—To a solution of 2 ml. (40 mmoles) of iodine monochloride in 10 ml. of 3 *N* HCl, a solution of 2.9 g. (20 mmoles) of 5-isoquinolinol[5] in 20 ml. of 3 *N* HCl was added rapidly, and the mixture was stirred at room temperature for 19 hr. The resulting precipitate (7.8 g.) was filtered, washed with 3 *N* HCl, dried, and dissolved in 35 ml. of pyridine and 18 ml. of acetic anhydride. The solution was kept at room temperature for 30 min. After evaporation to dryness *in vacuo* the residue (10.8 g.) was dissolved in 300 ml. of CH_2Cl_2. This solution was extracted four times with 100-ml. portions of water, dried (Na_2SO_4), and evaporated to dryness *in vacuo*. A red-brown product was obtained, which was absorbed on a column of Florisil from a CH_2Cl_2 solution. The column was eluted successively with CH_2Cl_2 and ether, and the combined eluates were evaporated to dryness under reduced pressure to give 5.1 g. of solid residue which was dissolved in CH_2Cl_2. This solution was washed with a 5% aqueous solution of sodium thiosulfate and then with water, and dried (Na_2SO_4). Evaporation of the solvent and crystallization of the residue from CH_2Cl_2–ether gave 4.64 g. (53%) of pure XII: m.p. 187.5°; λ_{max}, mμ (ϵ), 224 (24,000), 250 (31,250), 294 (5800), 305 (6000), 320 (4950), 333 (5100); ν_{max} 1775, 1182 cm.$^{-1}$; δ 9.36 (1H, s, C-1 H), 8.62 (1H, d, C-3 H), 8.50 (1H, s, C-7 H), 7.43 (1H, d, C-4 H), 2.51 (3H, s, CH_3).

Anal. Calcd. for $C_{11}H_7I_2NO_2$: C, 30.09; H, 1.61; N, 3.19. Found: C, 30.49; H, 1.82; N, 3.19.

6,8-Diiodo-5-isoquinolinol (IV).—With stirring, 16.2 g. (37 mmoles) of XII was added to 200 ml. of warm dimethylformamide. The resulting solution was cooled in an ice–water bath and treated with 200 ml. of 1 *N* NaOH. The reaction mixture was stirred for 3 hr. and filtered to remove a small amount of insoluble material. The filtrate was adjusted to pH 6.1 with 3 *N* HCl. The resulting precipitate was collected by filtration, washed with water, and dried in a desiccator to afford 14.5 g. (100%) of IV, m.p. 180–185° dec., as a tan powder: λ_{max}^{EtOH}, mμ (ϵ), 220 (sh) (37,750), 255 (31,500), 340 (6200).

Anal. Calcd. for $C_9H_5I_2NO$: I, 63.94; N, 3.54. Found: I, 63.71; N, 3.55.

8-Iodo-5-isoquinolinol (X).—A solution of 23 ml. (0.44 mole) of iodine monochloride in 100 ml. of cold ethanol was added to a suspension of 29 g. (0.2 mole) of 5-isoquinolinol[5] in ethanol with stirring and ice cooling. A clear solution was obtained which soon (*ca.* 20 min.) became turbid. After stirring the reaction mixture at 5° for 5 hr., the resulting solid material was filtered to give 19.2 g. (31%) of 8-iodo-5-isoquinolinol hydrochloride, m.p. 212–212.5° dec. A sample was recrystallized from methanol: m.p. 213–213.5°; λ_{max}^{MeOH}, mμ (ϵ), 248 (27,900), 268 (16,200), 304 (sh) (3800), 317 (sh) (4150), 333 (4650), 363 (sh) (3500).

Anal. Calcd. for $C_9H_6INO \cdot HCl$: C, 35.15; H, 2.30; N, 4.56. Found: C, 35.34; H, 2.46; N, 4.62.

The corresponding free base melted at 138–139°: λ_{max}, mμ (ϵ), 249 (40,500), 305 (sh) (4000), 321 (4950), 331 (5200).

Anal. Calcd. for C_9H_6INO: C, 40.20; H, 2.32; N, 5.42. Found: C, 39.88; H, 2.23; N, 5.17.

8-Iodo-5-isoquinolinol Acetate (XI).—To a solution of 4.8 g. (17.5 mmoles) of X in 50 ml. of pyridine, 25 ml. (26.5 mmoles) of acetic anhydride was added. The yellow solution became turbid within 10 min., and after stirring for 1.5 hr. at room temperature, the mixture was evaporated to dryness under high vacuum (bath temperature 30°). The residue was dissolved in CH_2Cl_2, and the resulting solution was washed twice with water, dried over Na_2SO_4, and evaporated to dryness to give 5.36 g. of solid. Crystallization from CH_2Cl_2–hexane yielded 4.65 g. (85%) of XI: m.p. 175.5–176° dec.: λ_{max}, mμ (ϵ), 227 (29,500), 243 (38,500), 272 (5800), 280 (5750), 297 (sh) (4000), 310 (2700), 325 (3300); ν_{max} 1775, 1190 cm.$^{-1}$; δ 9.23 (1H, s, C-1 H), 8.57 (1H, d, C-3 H), 7.96 (1H, d, C-6 H), 7.60 (1H, d, C-7 H), 7.52 (1H, d, C-4 H), 2.51 (3H, s, CH_3).

Anal. Calcd. for $C_{11}H_8INO_2$: C, 42.20; H, 2.57; I, 40.53; N, 4.47. Found: C, 42.24; H, 2.70; I, 40.81; N, 4.44.

8-Isoquinolinol (VIII).—A solution of 9.39 g. (30 mmoles) of VI in 200 ml. of ethanol and 60 ml. of 1 *N* NaOH was left for 2 hr. at room temperature and then hydrogenated for 1 hr. in the presence of 5 g. of Raney nickel at atmospheric pressure. The catalyst was removed by filtration, and the filtrate was evaporated to dryness *in vacuo* at room temperature. The residue was dissolved in 100 ml. of water and the pH of the solution was adjusted to 6.9. A precipitate was obtained which was filtered, washed with water, and dried to give 3.8 g. of crude product. This material was dissolved in 56 ml. of hot 1 *N* HCl, and 8 ml. of concentrated HCl was added. Cooling afforded 4.2 g. (77%) of 8-isoquinolinol hydrochloride, m.p. 240–242°, as yellow crystals. A sample, crystallized from ethanol–ether, was analyzed: m.p. 240–242°; λ_{max}, mμ (ϵ), 244 (20,250), 310 (2300), 335 (sh) (2000), 379 (3550).

Anal. Calcd. for $C_9H_7NO \cdot HCl$: N, 7.71. Found: N, 7.54.

A solution of 50 mg. of the hydrochloride in water was adjusted to pH 6.9 with 1 *N* NaOH. The precipitate which formed was filtered and washed with water. After drying in a desiccator, the crude material was sublimed twice under high vacuum to give pure VIII: m.p. 210–211°; λ_{max}, mμ (ϵ), 233 (24,100), 294 (sh) (3000), 332 (5200).

Anal. Calcd. for C_9H_7NO: C, 74.47; H, 4.86; N, 9.65. Found: C, 74.68; H, 4.90; N, 9.61.

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