Isolation and Characterization of Urinary Metabolites of Benzquinamide and Benzquinamide Alcohol

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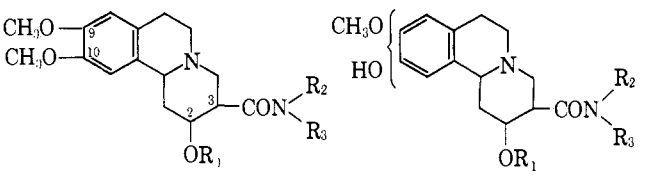
Unchanged benzquinamide and six crystalline metabolites isolated from the urine of dogs treated with benzquinamide were characterized. Five other metabolites were recovered as crude preparations and related by chemical transformations to the crystalline metabolites. Evidence for six more metabolites of unknown structure was obtained. The chemical nature of the metabolites demonstrated that benzquinamide is metabolized by at least three pathways: (a) O-deacetylation, (b) O-demethylation and conjugation, and (c) N-dealkylation (one and two groups). The same series of 2-acetoxy and 2-hydroxy metabolites was formed from benzquinamide in both man and the dog. A corresponding series of 2-hydroxy metabolites was formed from benzquinamide alcohol in man. Conjugates in the urine were demonstrated by enzymatic hydrolysis or acid hydrolysis to O-demethyl metabolites. In either the dimethoxy series or the conjugated O-demethyl series, the N-deethyl compounds appeared to be the major metabolites in the urine.

Benzquinamide,[1] 2-acetoxy-3-diethylcarbamoyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11bH-benzo[*a*]quinolizine (**1**), is a new benzoquinolizine compound[2] of interest as a psychotherapeutic agent.[3] As a part of a study of the metabolism of benzquinamide,[4] the chemical nature of the urinary metabolites formed in man and in the dog was determined. The human urinary metabolites of the pharmacologically active benzquinamide alcohol (**2**)[2] were also investigated.

The examination of paper chromatograms of crude extracts of various urines collected from human subjects or from dogs treated with benzquinamide disclosed a number of zones, the intense fluorescence of which indicated that these substances were metabolites of benzquinamide (Fig. 1–4, Table I). Earlier observations from the chemical synthesis of benzquinamide and related compounds[2b] had shown that the color of the characteristic fluorescence developed by these compounds on extended exposure of their paper chromatograms to ultraviolet light[5] could be correlated with the functional group at C-2: yellow fluorescence for 2-acetoxy compounds and blue fluorescence (slowly turning yellow) for 2-hydroxy compounds. The paper chromatograms of extracts of benzquinamide urines contained both yellow-fluorescent and blue-fluorescent zones, suggesting the presence of 2-acetoxy and 2-hydroxy metabolites, while extracts of benzquinamide alcohol urines exhibited only blue-fluorescent (2-hydroxy) metabolite zones. Methanolysis (deacetylation) and acetylation experiments carried out on crude extracts or on partially purified fractions (Table II) followed by paper chromatographic comparison of the products and starting materials established the relationship between several pairs of yellow- and blue-fluorescent zones as the C-2 acetates and alcohols, respectively, of the same benzoquinolizine moieties. Similarly, methylation experiments with diazomethane followed by paper chromatographic comparison of the products with untreated samples related some phenolic zones (located in the chromatograms by their color reaction with *p*-nitrobenzenediazonium reagent) to certain fluorescent zones, demonstrating the occurrence of several pairs of metabolites differing in the degree of O-methylation. The fractionation of the chloroform extract of alkalinized urine from benzquinamide-treated dogs by a combination of column chromatography and preparative paper chromatography led to the isolation of crystalline unchanged benzquinamide (**1**) and six crystalline metabolites, **2**, **3**, **4**, **5**, **6**, and **10**.



A B

A	B	
1	**7**	$R_1 = CH_3CO$; $R_2, R_3 = C_2H_5$
2	**8**	$R_1 = H$; $R_2, R_3 = C_2H_5$
3	**9**	$R_1 = CH_3CO$; $R_2 = H$; $R_3 = C_2H_5$
4	**10**	$R_1, R_2 = H$; $R_3 = C_2H_5$
5	**11**	$R_1 = CH_3CO$; $R_2, R_3 = H$
6	**12**	$R_1, R_2, R_3 = H$

Characterization or identification of these compounds depended on elemental analysis (when possible), on ultraviolet and infrared absorption spectra, and on paper chromatographic comparisons with synthetic compounds. Five O-demethyl metabolites—**7**, **8**, **9**, **11**, and **12**—were not isolated in the pure state but could be readily detected in paper chromatograms and chemically related to the corresponding dimethoxybenzoquinolizines (A) by the methylation experiments mentioned previously. Three pairs of unidentified zones possessing the same type of fluorescence as the A compounds are considered to be drug-related metabolites, because methanolysis converted three yellow-fluorescent zones (a, c, e) to slower moving blue-fluorescent zones

(1) Quantril®.

(2) (a) K. F. Finger, A. Weissman, and J. R. Tretter, *The Pharmacologist*, **3**, 75 (1961); (b) J. R. Tretter, J. G. Lombardino, K. F. Finger, and A. Weissman, Abstracts of Papers, 140th National Meeting of American Chemical Society, Chicago, Ill., Sept., 1961, p. 4O; (c) A. Weissman and K. F. Finger, *Biochem. Pharmacol.*, **11**, 871 (1962); (d) A. Scriabine, A. Weissman, K. F. Finger, C. S. Delahunt, J. W. Constantine, and J. A. Schneider, *J. Am. Med. Assoc.*, **184**, 276 (1963).

(3) M. E. Smith, *Diseases Nervous System*, **24**, 116 (1963); J. E. Overall, L. E. Hollister, J. L. Bennett, Jr., J. Shelton, and E. M. Caffey, Jr., *Current Therap. Res.*, **5**, 335 (1963).

(4) E. H. Wiseman, E. C. Schreiber, and R. Pinson, Jr., *The Pharmacologist*, **4**, 156 (1962); *Biochem. Pharmacol.*, in press.

(5) Subsequent photodecomposition products formed by the benzoquinolizine zones on the paper chromatograms were responsible for the visible fluorescence color observed. Compounds **1**, **2**, **3**, **4**, **5**, and **6** all exhibited the same type of fluorescence spectrum (uncorrected fluorescence λ_{max} 330 mμ, activation λ_{max} 280 mμ in 0.1 *N* HCl) as tetrabenazine [G. P. Quinn, P. A. Shore, and B. B. Brodie, *J. Pharmacol. Exptl. Therap.*, **127**, 103 (1959)].

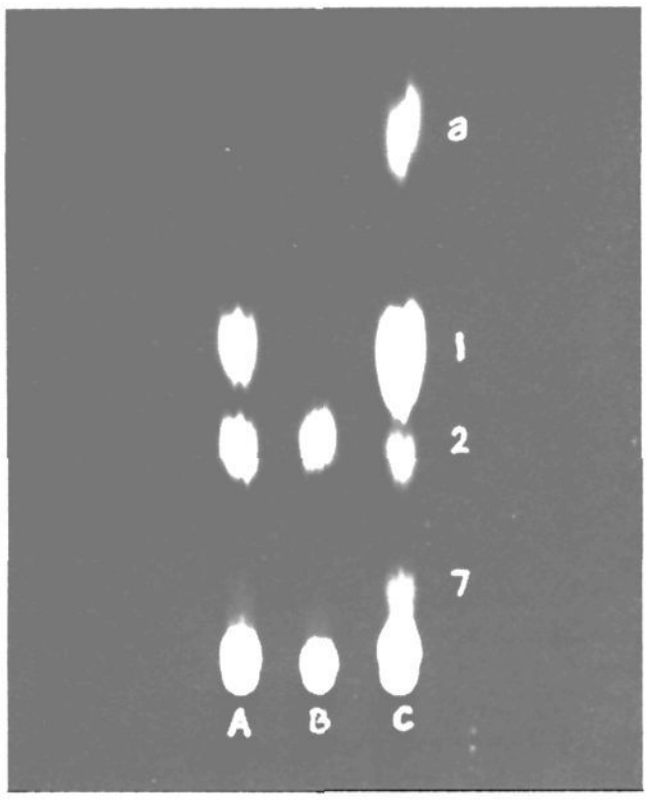
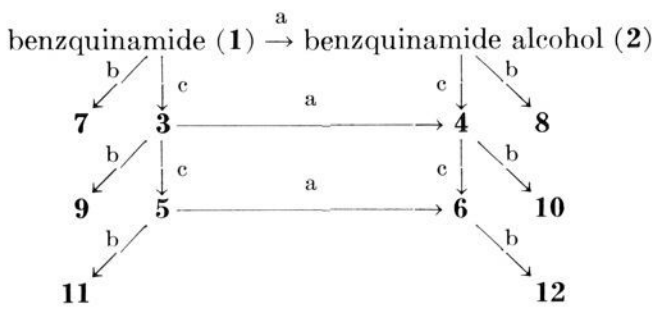


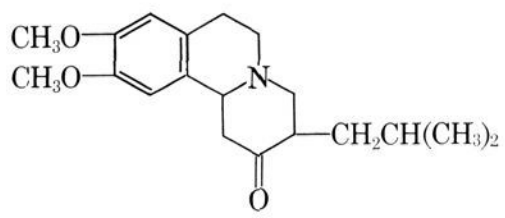
Fig. 1.—Fluorescent paper chromatogram of benzquinamide metabolites in system I (2 passes). Chloroform solution applied at A, extract of human-benzquinamide urine; at B, extract of human-benzquinamide alcohol urine; at C, extract of dog-benzquinamide urine; relative R_f values: a, 1.00; **1**, 0.61; **2**, 0.42; **7**, 0.14.

(b, d, f, respectively), a transformation associated with deacetylation at C-2. The major metabolites observed in extracts of human- and dog-benzquinamide urines were essentially the same except for differences in the minor unidentified fluorescent zones.



The metabolites identified in the urine demonstrated that benzquinamide is metabolized by at least three pathways: (a) deacetylation of the 2-acetoxy group, (b) demethylation of the 9- or 10-methoxy group and conjugation, and (c) deethylation of the 3-diethylcarbamoyl group.[6] The N-deethyl compounds appeared to be the major urinary metabolites with the benzoquinolizine structure in either the dimethoxy series (A) or in the conjugated O-demethyl series (B).[7]

(6) A number of urinary metabolites and conjugates have been reported for tetrabenazine by A. Pletscher, A. Brossi, and K. F. Gey [*Intern. Rev. Neurobiol.*, **4**, 275 (1962)]. Only two metabolites, the two epimeric alcohols from reduction of the 2-keto group, have been identified by comparison with synthetic compounds.



tetrabenazine

(7) Approximately 3% (range, 1.5–6.6%) of an oral dose (100 to 300 mg.) of benzquinamide in man is found as unchanged drug in the urine [B. Cahn, L. S. Brahen, E. H. Wiseman, and R. Pinson, Jr., *Current Therap. Res.*, **5**, 301 (1963)]. The amount of metabolite **3** excreted in the urine is estimated to be about 3–6 times that of unchanged drug.

TABLE I
BENZQUINAMIDE AND METABOLITES

No.	Compound	Chromatographic system[a]
1	Benzquinamide	I, 3 passes[b]; IV, 20 hr.
2	Benzquinamide alcohol	I, 3 passes; IV, 20 hr.
3	N-Deethylbenzquinamide	II; V, 2.5 hr.
4	N-Deethylbenzquinamide alcohol	II; V, 2.5 hr.
5	N,N-Bisdeethylbenzquinamide	III, 3 passes; VI, 2.5 hr.
6	N,N-Bisdeethylbenzquinamide alcohol	III, 3 passes; VI, 18 hr.
7	O-Demethylbenzquinamide	II
8	O-Demethylbenzquinamide alcohol	II; I, 16 hr.
9	N-Deethyl-O-demethylbenzquinamide	III, 3 passes
10	N-Deethyl-O-demethylbenzquinamide alcohol	III, 3 passes
11	N,N-Bisdeethyl-O-demethylquinamide	III, 3 passes
12	N,N-Bisdeethyl-O-demethylbenzquinamide alcohol	III, 3 passes
a	Unidentified metabolite (2-acetoxy)	I, 2 passes
b	Alcohol of a (2-hydroxy)	I, 2 passes
c	Unidentified metabolite (2-acetoxy)	V, 2.5 hr.
d	Alcohol of c (2-hyroxy)	V, 2.5 hr.
e	Unidentified metabolite (2-acetoxy)	III, 3 passes; VI, 2.5 hr.
f	Alcohol of e (2-hydroxy)	VI, 18 hr.

[a] Systems, using formamide–acetone (2:3) treated paper: I, hexane–benzene–diethylamine (27:9:4), formamide-saturated; II, benzene–diethylamine (9:1), formamide-saturated; III, benzene–chloroform–diethylamine (13:6:1), formamide-saturated; *cf.* D. Waldi, *Arch. Pharm.*, **292**, 206 (1959). Relative R_f values are given in Fig. 1–4. The following order of decreasing rate of migration was observed in the diethylamine systems (I, II, III): a, b, **1**, **2**, **7**, **8**, **3**, **4**, **9**, **5**, e, **10**, **6**, **11**, and **12**. Supplementary formamide-saturated systems, using pH 8 buffered paper: IV, hexane, 20 hr. (relative R_f values: **1**, 1.00; **2**, 0.65); V, benzene, 2.5–4 hr. for **3** to **4** region (relative R_f values for 2.5-hr. run: **3**, 1.00; **4**, 0.35; **9**, 0.15; **5**, 0.11); VI, benzene–chloroform (1:1), 2.5–18 hr. for **5** to **12** region (relative R_f values for 2.5-hr. run: **5**, 1.00; **9**, 0.83; e, 0.46; **10**, 0.24; **6**, 0.17).
[b] One pass is defined as the time required for the descending solvent front to reach 20 mm. above the lower edge of the suspended sheet, which was 40-cm. long.

Dealkylation from a carboxamide nitrogen is well documented in the special instance of imide nitrogen such as the dealkylation of N-alkylbarbiturates or the demethylation of 5-ethyl-3-methyl-5-phenylhydantoin.[8] Metabolic removal of alkyl groups from aliphatic tertiary amides have been described more recently; *e.g.*, the demethylation in the rat of (−)-cotinine to (−)-demethylcotinine[9a] and the monodealkylation of N,N-dialkylcarbamate esters by rat-liver microsomes.[9b] The metabolic dealkylation sequence of tertiary amide → secondary amide → primary amide, as in the formation of **5** and **6** from benzquinamide, is not an unexpected series of transformations in view of the deethylation of N-ethylbenzilamide to benzilamide in the dog.[10]

The structures assigned to the N-deethyl metabolites,

(8) R. T. Williams, "Detoxication Mechanisms," 2nd Ed., John Wiley and Sons, Inc., New York, N. Y., 1959, pp. 581, 604–605.

(9) (a) H. McKennis, Jr., L. B. Turnbull, S. L. Schwartz, E. Tamaki, and E. R. Bowman, *J. Biol. Chem.*, **237**, 541 (1962); (b) E. Hodgson and J. E. Casida, *Biochem. Pharmacol.*, **8**, 179 (1961).

(10) J. R. Albert, G. C. Boxill, and J. H. Weikel, Jr., *J. Pharmacol. Exptl. Therap.*, **131**, 85 (1961).

TABLE II
CHROMATOGRAPHIC FRACTIONS OF DOG-URINE BENZQUINAMIDE METABOLITE MIXTURE[a]

Fraction	Volume, ml.	Developer	Residue wt.,[b] g.	Metabolite content[c]
I	125	50% $CHCl_3$	0.09, oil	a
II	425	50% $CHCl_3$	0.74, oil	1, 2, a, b
III	375	50–75% $CHCl_3$	0.19, g. oil	1, 2
IV	500	75% $CHCl_3$	1.26, cryst.	3
V	275	75% $CHCl_3$	0.58, oil	3, 4, 7, 8, c
VI	1675	75% $CHCl_3$–10% MeOH	0.77, oil	4, 7, 8, c
VII	200	10% MeOH	0.75, amorph.	5, 6, 9, 10, e
VIII	250	15% MeOH	0.05, oil	5, 6, 9, 10, 11, 12, e
IX	500	20–40% MeOH	0.05, cryst.	9, 10, 11, 12, origin

[a] Column (length, 46.5 cm.; diameter, 5.2 cm.): neutral Woelm, grade III, alumina (395 g.); charge: benzene solution (160 ml.) of crude metabolite gum (8 g.); developers: 0.5-l. volumes of 5, 10, and 25% chloroform–benzene; 1-l. volumes of 50 and 75% chloroform–benzene; 1 l. of chloroform; 0.25-l. volumes of 1, 2, 10, 15, 20, and 40% (twice) methanol–chloroform. [b] Residue weight after evaporation of the fraction and not the weight of metabolites in residue (except for **3**). [c] The column cuts were examined for metabolite content by paper chromatography in system II, with additional resolution in other systems when necessary. The eluate up to 50% benzene–chloroform contained no fluorescent metabolites, while the remaining eluates (25-ml. cuts) were combined into fractions I to IX.

3 and **4**, on the basis of elemental analysis, methoxyl group determination, and spectral data were verified by deacetylating **3** to **4** and establishing the identity of the latter compound with totally synthetic N-deethylbenzquinamide alcohol.[2b] Corresponding physical and chemical data support the structure, N,N-bisdeethylbenzquinamide, for **5**. Since **5** gave **6** on deacetylation, the latter is N,N-bisdeethylbenzquinamide alcohol. The structure assignments for **7**–**10** based on the results of methylation and deacetylation experiments of the type mentioned earlier[11] were strengthened by the isolation of several crystalline methylation products, which were identical with authentic samples: **1** from **7** (fraction V), **3** from **9**, **4** from **10**. The phenolic metabolites are considered to be O-monodemethyl compounds for three reasons. (1) Dihydroxy derivatives, like the catecholamines, would be unstable under the alkaline conditions used for extraction and paper chromatography. (2) The shift in λ_{max} (280 to 295 mμ region) in going from acid to alkaline solution for **10** (shift = 7 mμ; ϵ in HCl: ϵ in NaOH = 0.93) resembled that of 3-hydroxy-4-methoxyphenylethylamine (shift = 8 mμ; ϵ in HCl: ϵ in NaOH = 0.91) but not that of dopamine (shift = 11.5 mμ; ϵ in HCl: ϵ in NaOH = 0.68). (3) A paper chromatographic comparison showed that **8** (enriched sample prepared by subjecting fraction V to methanolysis) was the same as one of the two O-monodemethylbenzquinamide alcohols derived chemically from authentic benzquinamide alcohol.[12] The detection of O-demethyl compounds in extracts of untreated urines suggested

(11) Since **7** (fraction V) → **1** and **8** → **2** on methylation, while **7** → **8** and **1** → **2** on deacetylation, **7** and **8** are O-demethylbenzquinamide and O-demethylbenzquinamide alcohol, respectively. Similarly, **9** → **3** and **10** → **4** on methylation, while **9** → **10** and **3** → **4** on deacetylation. Since structures of **3** and **4** have been determined, **9** and **10** are N-deethyl-O-demethylbenzquinamide and N-deethyl-O-demethylbenzquinamide alcohol, respectively.

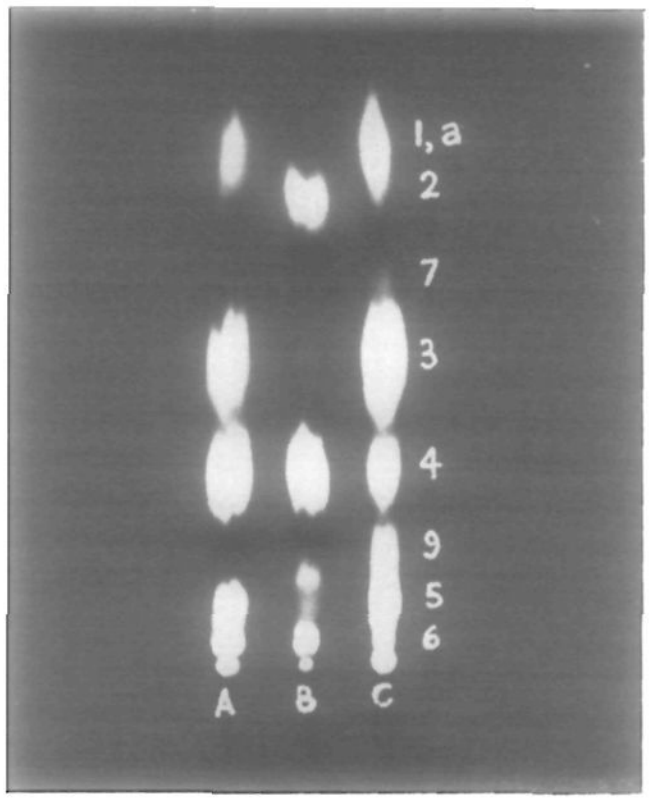


Fig. 2.—Fluorescent paper chromatogram of benzquinamide metabolites in system II. Same solutions as in Fig. 1; R_f values: **2**, 0.77; **7**, 0.64; **3**, 0.51; **4**, 0.34; **9**, 0.17; **5**, 0.11; **6**, 0.05.

the occurrence of conjugated metabolites, the presence of which was corroborated by the formation of B by either acid hydrolysis or enzymatic hydrolysis of urines pre-extracted exhaustively with chloroform at pH 10. The absence of benzquinamide alcohol in similar acid hydrolysates indicated that conjugation did not occur at the 2-hydroxy group. The intact conjugates of **9** and **10** were detected in paper chromatograms of concentrated human-benzquinamide urine by acid hydrolysis of the eluted zones to **10**.

The rate of enzymatic dealkylation of aromatic methyl ethers[13a] is apparently increased by *para* electron-attracting groups (−R, −I effects), which can enhance the electron-repelling effect (+R) of the aromatic methoxy groups.[13b] Such a correlation would account for the greater rate of enzymatic dealkylation of *p*-O-methylcatechols, like 3-hydroxy-4-methoxyacetophenone, 3-hydroxy-4-methoxyphenylmethylcarbinol, paranephrine, and norparanephrine, as compared to the corresponding *m*-O-methylcatechols,[13c] as well as the metabolic dealkylation of the 6-methoxy rather than the 4-methoxy group in griseofulvin.[13d] Both O-monomethyldopamines are demethylated *in vitro* at the same rate (about one-half that of paranephrine or norparanephrine),[13c] suggesting that the aminoethyl group has little influence. In contrast, attaching a *p*-aminomethyl group (−I effect) to anisole increased the demethylation rate about eightfold.[13a] Since the 9-methoxy group in the benzoquinolizine A series is part of a similar benzylamine system and would be

(12) J. R. Tretter, unpublished synthesis. The location of the phenolic OH in the two O-monodemethylbenzquinamide alcohols (at C-9 or C-10) has not been established. For synthetic **8** the shift in λ_{max} from acid to alkaline solution is 8 mμ (ϵ in HCl: ϵ in NaOH = 0.95).

(13) (a) J. Axelrod, *Biochem. J.*, **63**, 634 (1956). (b) The substituent effect on this oxidative O-demethylation by liver microsomes has a qualitative similarity to the effect of substituents on nucleophilic aromatic substitution (C. K. Ingold, "Structure and Mechanism in Organic Chemistry," Cornell University Press, Ithaca, N. Y., 1953, pp. 802–811). The resultant partial positive charge on the ether oxygen could be significant in the mechanism of dealkylation by O-demethylase [*cf.* R. E. McMahon, H. W. Culp, J. Mills, and F. J. Marshall, *J. Med. Chem.*, **6**, 343 (1963)]. (c) J. W. Daly, J. Axelrod, and B. Witkop, *J. Biol. Chem.*, **235**, 1155 (1960). (d) M. J. Barnes and B. Boothroyd, *Biochem. J.*, **78**, 41 (1961).

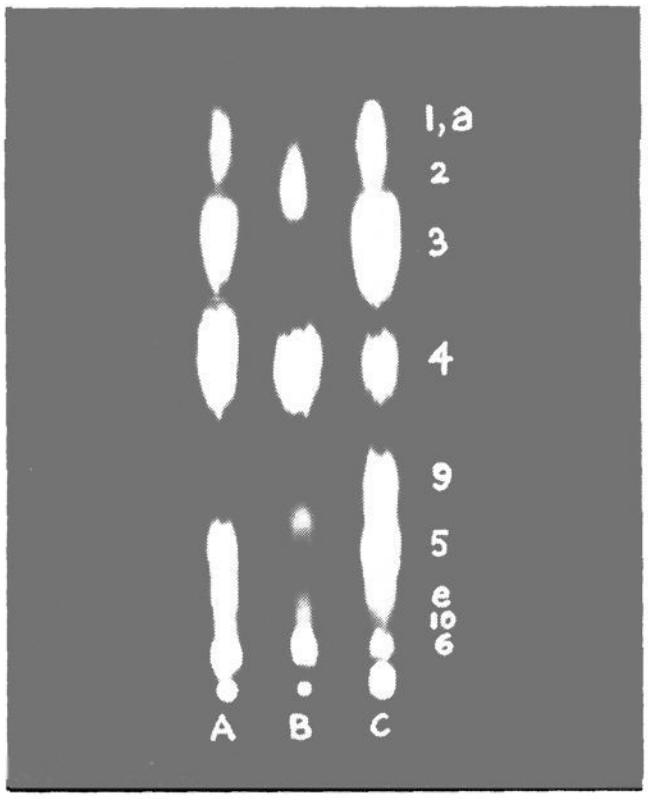


Fig. 3.—Fluorescent paper chromatogram of benzquinamide metabolites in system III. Same solutions as in Fig. 1; R_f values: **2**, 0.84; **3**, 0.75; **4**, 0.54; **9**, 0.34; **5**, 0.24; e, 0.18; **6**, 0.07.

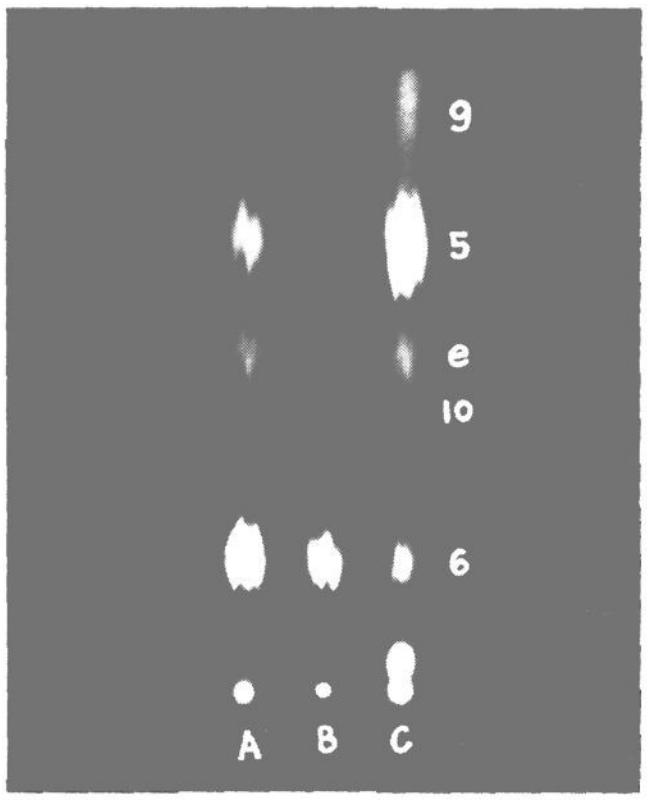


Fig. 4.—Fluorescent paper chromatogram of benzquinamide metabolites in system III (3 passes). Same solutions as in Fig. 1; relative R_f values: **9**, 1.00; **5**, 0.77; e, 0.58; **10**, 0.48; **6**, 0.23.

expected to undergo a faster metabolic dealkylation than the 10-methoxy group, the phenolic OH in the B series is tentatively placed at C-9.

N-Deethylbenzquinamide (**3**) was inactive when tested for the disruption of conditioned avoidance behavior in rats[14] in contrast to the potent effect of benzquinamide.[2c]

Experimental

Materials.—Urine samples collected from human subjects treated orally (100–400 mg./kg.) with benzquinamide or benzquinamide alcohol for the metabolism studies[4] were combined for each drug for the isolation experiments. Urines from four dogs on an oral chronic regimen of benzquinamide (40 mg./kg., 6 days weekly for 4 weeks) were pooled. All urines were refrigerated as freeze-dried solids and reconstituted in water at a higher concentration for subsequent extractions.

Paper Chromatography.—Chloroform solutions[15] were chromatographed on formamide–acetone(2:3)-treated paper (Whatman No. 4) placed in descending systems using formamide-saturated solvent solutions as the moving phase (Table I, Fig. 1–4). Benzquinamide and related 2-acetoxy metabolites were not deacetylated by basic systems containing diethylamine (I, II, III; see Table I, footnote *a*). Chromatographic results in these solvent systems were confirmed in formamide-saturated solvent systems omitting the diethylamine but using pH 8 buffered paper.

Isolation.—Crude metabolite mixtures (gums) were prepared by extracting the urines 1–3 times with equal volume of chloroform at pH 10 and evaporating the dried (Na_2SO_4) extracts.[16] Initial fractionation was achieved by column chromatography on neutral alumina (Woelm, grade III). Benzquinamide and related 2-acetoxy metabolites were not deacetylated by this adsorbent in control columns. Table II summarizes the preliminary resolution of the crude gum derived from dog-benzquinamide urine into fractions I–IX. The isolation of metabolites from these nine fractions either by direct crystallization as for **3** (fraction IV) or after further column or preparative paper chromatography is described under the individual metabolites.

Unchanged Benzquinamide (1).—Paper chromatographic comparison of crude extracts with authentic **1** demonstrated the presence of unaltered drug in human- and dog-benzquinamide urines (Fig. 1). Crystalline **1** was recovered: (1) from fraction II by rechromatography on alumina (20 g.), eluting with benzene (75 ml.), and recrystallizing from benzene–hexane, 56-mg. yield; and (2) from fraction III by the preparative paper chromatograms described in the next section, 60-mg. yield, m.p. 126–128°, λ_{max} 284 mμ (ε 3880) in 0.01 *N* HCl–methanol, infrared spectrum identical with that of benzquinamide [authentic **1**, m.p. 130–131°, λ_{max} 284 mμ (ε 3760), ν_{max} (cm.$^{-1}$): 1730 (acetoxy C=O), 1629 (amide C=O) in KBr].

Benzquinamide Alcohol (2).—This compound was detected in paper chromatograms of crude extracts of human- and dog-benzquinamide urines, as well as in extracts of human-benzquinamide alcohol urine (Fig. 1). Fraction III was chromatographed on four sheets (16.2 × 40 cm.) of paper in system I (3 passes). Crystalline **2** was recovered by eluting the proper band with 0.01 *N* HCl and recrystallizing from benzene–hexane; yield, 5 mg.; m.p. 136–137°; λ_{max} 285 mμ (ε 3910); infrared spectrum identical with that of benzquinamide alcohol [authentic **2**, m.p. 132–134°; λ_{max} 284.5 mμ (ε 3660); ν_{max} (cm.$^{-1}$): 3300 (OH), 1610 (amide C=O)].

N-Deethylbenzquinamide (3).—The solid obtained from the evaporation of fraction IV was crystallized by triturating with benzene, yielding 1.26 g. of **3**, m.p. 157–160°. For analysis a sample was chromatographed on alumina and recrystallized from benzene–hexane, m.p. 160–161°; λ_{max} 284.5 mμ (ε 3570); ν_{max} (cm.$^{-1}$): 3268 (NH), 1736 (acetoxy C=O), 1642 (amide C=O), 1563 (secondary amide NH).

Anal. Calcd. for $C_{20}H_{28}N_2O_5$: C, 63.81; H, 7.50; N, 7.44; CH_3O (two), 16.49. Found: C, 64.04; H, 7.59; N, 7.24; CH_3O, 16.43.

Crystalline **3** was also isolated from human-benzquinamide urine (Fig. 2) by the same procedure given in Table II; yield, 10 mg. from 23 l.; m.p. 157–160°; identical infrared spectrum.

N-Deethylbenzquinamide Alcohol (4). A. From 3.—A solution of **3** (250 mg.) in 1 *N* methanolic NaOH (63 ml.) kept 15 min. at room temperature was diluted with water (2 volumes), adjusted to pH 10, and extracted twice with equal volumes of chloroform. The residue left from evaporating the chloroform solution crystallized when triturated with acetone. The suspension was diluted with ether and filtered, yielding 167 mg. of **4**, m.p. 146–148°. For analysis a sample was recrystallized from acetone, m.p. 150–151°; λ_{max} 284.5 mμ (ε 3640); ν_{max} (cm.$^{-1}$): 3344 (NH, OH), 1653 (amide C=O). This product was identical (m.m.p. 148–150°, infrared spectrum, paper chromatography) with synthetic 3-ethylcarbamoyl-2-hydroxy-9,10-dimethoxy-1,-

(14) A. Weissman, unpublished observation.

(15) Impurities in crude extracts interfering with the paper chromatography were removed by washing aqueous solutions of the extract residues with heptane at pH 2 and 7 and recovering the metabolites by extraction into chloroform at pH 10.

(16) Extraction of a concentrated (7-fold) human-benzquinamide urine with equal volumes of chloroform consecutively at pH 2, 7, and 10 showed that benzquinamide (**1**), benzquinamide alcohol (**2**), and the more lipophilic metabolites were removed by chloroform at pH 2; **3** and **4** were partly extracted at pH 2; **5** and **6** were extracted to a slight extent at pH 7; the unextracted portion of these metabolites and other less lipophilic ones were completely removed at pH 10.

2,3,4,6,7-hexahydro-11bH-benzo[*a*]quinolizine (m.p. 148–150°) prepared by Tretter.[2b]

Anal. Calcd. for $C_{18}H_{26}N_2O_4$: C, 64.65; H, 7.84; N, 8.38; CH_3O (two), 18.56. Found: C, 65.07; H, 7.73; N, 8.54; CH_3O, 18.65.

B. From Urines.—Crystalline **4** was isolated: (1) from fraction V by preparative paper chromatography (system II, 2 passes), 10-mg. yield, m.p. 146–149°; and (2) from human-benzquinamide alcohol urine (Fig. 3) by the same method described in Table II, 3-mg. yield from 21 l., m.p. 146–147°. Both products proved to be identical with **4** from part A by paper chromatographic comparison, by comparison of infrared spectra, and by acetylation (acetic anhydride, concentrated H_2SO_4 catalyst) to **3** (latter demonstrated by resolution of the reaction product on paper).

N,N-Bisdeethylbenzquinamide (5).—Metabolite **5** was present in human- and dog-benzquinamide urines but absent in benzquinamide alcohol urine (Fig. 4). Fraction VII (260 mg.) was chromatographed in system III (3 passes) using 5–10 mg. per sheet of paper. The yellow-fluorescent **5** band was cut out and stirred 20 min. with 0.01 *N* HCl (650 ml.). The eluate was extracted twice with 0.5 volumes of chloroform at pH 10. The dried extract on evaporation gave a crystalline residue, which was recrystallized from ether; yield, 71 mg. For analysis a sample was recrystallized from acetone, m.p. 193–195° dec.; λ_{max} 284.5 mμ (ε 3380); ν_{max} (cm.$^{-1}$): 3448, 3289, 3165 (NH, OH), 1724 (acetoxy C=O), 1681 (amide C=O).

Anal. Calcd. for $C_{18}H_{24}N_2O_5$: C, 62.05; H, 6.94; N, 8.04; CH_3O (two) 17.82. Found: C, 62.59; H, 7.21; N, 7.92; CH_3O, 17.67.

N,N-Bisdeethylbenzquinamide Alcohol (6).—Crystalline **6** was prepared by treating **5** (10 mg.) with 1 *N* methanolic NaOH (5 ml.) as described for **4**; yield, 4 mg.; m.p. 182–185° dec.; λ_{max} 285 mμ (ε 3430); ν_{max} (cm.$^{-1}$): 3448, 3333, 3185 (NH, OH), 1667 (amide C=O). This deacetylation product of **5** was identical paper chromatographically with a blue-fluorescent metabolite present in extracts of benzquinamide and benzquinamide alcohol urines (Fig. 4).

O-Demethylbenzquinamide (7).—A solution of fraction V (350 mg.) in methanol (1 ml.) treated with ethereal diazomethane (0.24 *M*, 90 ml.) was refrigerated overnight, kept 4 hr. at room temperature, and evaporated. The residue on paper chromatography (8 sheets, system I, 3 passes) exhibited zones corresponding to benzquinamide and benzquinamide alcohol. Crystalline **1** was recovered from the former zone by eluting with 0.01 *N* HCl; yield, 4 mg.; m.p. 125–128°; infrared spectrum identical with that of authentic benzquinamide. Spraying the paper chromatograms (system II) of untreated fraction V (**1** and **2** absent) with *p*-nitrobenzenediazonium solution and dilute Na_2CO_3 revealed two brownish violet zones, **7** (major one), migrating slightly faster than **3**, and **8**, having approximately the same R_f as **3** (Fig. 2). Both phenolic zones were absent in the chromatograms of the diazomethane-treated solution, indicating that **7** and **8** are O-demethylbenzquinamide and O-demethylbenzquinamide alcohol, respectively.

O-Demethylbenzquinamide Alcohol (8).—The content of **8** in fraction V was enriched by subjecting the latter to deacetylation (**7** → **8**) as described under **4**. Treating a part of the methanolysis product with diazomethane by the same method given in the preceding section demonstrated the conversion of **8** to **2**. Paper chromatographic comparison with the two authentic O-monodemethylbenzquinamide alcohols,[12] which differed in R_f value and the color produced with the *p*-nitrobenzenediazonium reagent (brownish violet color for the faster zone, brown color for the slower zone), showed that **8** was the same as the faster moving isomer.[17] Relative R_f values (system I, 16 hr.) are: metabolite **8** (violet zone), 1.00; synthetic **8** (O-demethylbenzquinamide alcohol, violet zone), 1.00; O-demethylbenzquinamide alcohol (brown zone), 0.69; **3**, 0.38; **4**, 0.20.

N-Deethyl-O-demethylbenzquinamide (9).—Spraying the paper chromatograms (system III, 3 passes) of fraction VII or of crude extracts of benzquinamide urines with the *p*-nitrobenzenediazonium reagent disclosed two brownish violet phenolic zones, **9** (major one) and **10** (Fig. 3 and 4). Crude **9** isolated from fraction VII (390 mg.) by preparative paper chromatography (system III, 3 passes) was treated with diazomethane, and crystalline **3** (4 mg.) was recovered from the chromatograms (system II) of the methylation product and identified by its infrared spectrum. For confirmation, part of the sample (2 mg.) was deacetylated to give 0.4 mg. of crystals, whose infrared spectrum was identical with that of **4**. The formation of **3** from **9** indicated that **9** is N-deethyl-O-demethylbenzquinamide. Methanolysis as described under **4** converted **9** to **10**, as shown by resolution of the reaction product on paper.

N-Deethyl-O-demethylbenzquinamide Alcohol (10). A. Acid Hydrolysis of Urines.—The spent aqueous phase from the chloroform extraction (pH 10) of dog-benzquinamide urine was concentrated *via* freeze drying to 1.5 l., adjusted to pH 0–0.3 (concentrated HCl), and heated at 100° for 1 hr. These hydrolytic conditions deacetylated the 2-acetoxy compounds, but neither **2** nor **4** was demethylated by similar treatment with 0.6 *N* to 1.2 *N* HCl. The hydrolysate was washed with chloroform (1 volume), adjusted to pH 10, and extracted with chloroform (1 volume). The concentrated extract (10 ml.), shown by paper chromatography to contain **8**, **10** (predominant zone), and **12**, was developed on a column of alumina (50 g.) with 100-ml. volumes of chloroform and 0.5%, 1% (twice), 2%, 5%, and 10% methanol–chloroform.[18] The 1% methanol–chloroform eluate on concentrating to 5 ml. gave crystalline **10**; yield, 54 mg.; m.p. 110–120° dec.; λ_{max} 287 mμ (ε 3580) in 0.01 *N* HCl–methanol; λ_{max} 294 mμ (ε 3870) in 0.01 *N* NaOH–methanol; ν_{max} (cm.$^{-1}$): 3521, 3333, 3279 (NH, OH), 1637 (amide C=O). A second crop of **10** (90 mg.) was recovered from the mother liquor. In a similar manner **10** was also isolated from human-benzquinamide urine (16 mg. from 22 l.) and from human-benzquinamide alcohol urine (7 mg from 21 l.).

B. Conversion to 4.—A solution of **10** (20 mg.) in methanol (0.5 ml.) treated with ethereal diazomethane (0.37 *M*, 50 ml.) was chromatographed on paper (system II). Eluting the band corresponding to **4** with 0.01 *N* HCl and recrystallizing the product from acetone yielded 2 mg., m.p. 147–148°; infrared spectrum identical with that of **4**.

O-Demethyl Metabolites 11 and 12.—The combined 5 and 10% methanol–chloroform eluates from the column for **10** were evaporated, and an aliquot of the residue was treated with diazomethane. Examination of the paper chromatogram (system III, 3 passes) of the product showed the formation of a new zone corresponding to **5** not present in an untreated aliquot and a more intense blue-fluorescent zone for **6**. The untreated sample contained a strong phenolic zone (unresolved mixture of **11** and **12**), which on the basis of the diazomethane reaction are N,N-bisdeethyl-O-demethylbenzquinamide and N,N-bisdeethyl-O-demethylbenzquinamide alcohol, respectively.[19] Relative R_f values (system III, 3 passes) are: **9**, 1.00; **6**, 0.23; **11** and **12**, 0.13. The phenolic zone corresponding to the **11** and **12** mixture was also detected in the same manner in fraction VIII (Table II).

Conjugates of O-Demethyl Metabolites. A. Enzymatic Hydrolysis of Urines.—The substrate was prepared by extracting human-benzquinamide urine (concentrated 4-fold) four times with equal volumes of chloroform at pH 10 to remove free O-demethyl metabolites, adjusting the aqueous phase to pH 5.2 with glacial acetic acid, adding penicillin G (1.2 mg./ml. as a preservative), and diluting to the original urine volume with pH 5.2 0.1 *M* acetate buffer. Aliquots (50 ml.) were incubated at 37° for 42 hr. with 0.6 ml. of Glusulase (Endo Laboratories, 100,000 units/ml. of glucuronidase and 50,000 units/ml. of sulfatase) or 400 mg. of β-glucuronidase (Mann Research Laboratories, 60,000 units/g.) and extracted with chloroform (1 volume) at pH 10. The residue from the evaporation of the dried extract was chromatographed in system II and in system III (3 passes) with appropriate controls, including the extract of substrate incubated without enzyme. The formation of **7–10** by enzymatic hydrolysis was shown clearly on the paper chromatograms. As expected, diazomethane treatment of the crude mixture of O-demethyl metabolites liberated by enzymatic hydrolysis formed the six A compounds, as demonstrated by resolution of the methylation product in systems I, II, and III. Metabolite **10** was also detected following enzymatic treatment of human-benzquinamide alcohol urine in the same manner.

(17) Synthetic **8**[12]: m.p. 176–180°; λ_{max} 286 mμ (ε 3840) in 0.01 *N* HCl–methanol; λ_{max} 294 mμ (ε 4060) in 0.01 *N* NaOH–methanol; ν_{max} (cm.$^{-1}$): 3378 (OH), 1610 (amide C=O).

(18) The combined chloroform and 0.5% methanol–chloroform eluates from this column on evaporation yielded a gum (80 mg.) containing **8**.

(19) Acid hydrolysis of concentrated urines did not completely deacetylate the 2-acetoxy metabolites, accounting for some **11** in the untreated aliquot; the latter also contained a trace of **6**.

B. Intact Conjugates.—The conjugates of **9** and **10** were located as weakly fluorescent bands in the paper chromatograms (ten 46.4 × 53.3 cm. sheets) of concentrated (20-fold) human-benzquinamide urine (0.5 ml./sheet) in a butanol–acetic acid–water (5:1:4) system by sectioning each chromatogram, combining corresponding bands, eluting the latter with 0.01 *N* HCl, acid hydrolysis of each eluate, and detection of the O-demethyl metabolite formed (**10**). Relative R_f values (16-hr. run) are: **10**, 1.00; **9**-conjugate, 0.72; 8-hydroxyquinoline glucuronide, 0.63; **10**-conjugate, 0.53.

Unidentified Metabolites.—Evidence that three unidentified yellow-fluorescent zones, a, c, and e, observed in paper chromatograms of extracts of dog-benzquinamide urine (Fig. 1 and 4) are drug-related metabolites was furnished by their methanolysis (deacetylation) to the slower moving blue-fluorescent zones, b, d, and f, respectively. R_f values are: system I—a, 0.38; b, 0.20; **1**, 0.18; **2**, 0.13. Relative R_f values are: system I (3 hr.)—a, off sheet; b, 1.00; **1**, 0.89; **2**, 0.63; system V (2.5 hr.)—**3**, 1.00; c, 0.85; **4**, 0.42; d, 0.26; system VI (18 hr.)—e, off sheet; **6**, 1.00; f, 0.83.

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Synthesis and Biological Evaluation of Water-Soluble 2-Boronoethylthio Compounds

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The radical-catalyzed addition of mercaptans to the double bond of dibutyl ethyleneboronate has been employed for the synthesis of several water-soluble boronic acids. Adducts have been obtained with mercaptoacetic acid, β-mercaptopropionic acid, mercaptosuccinic acid, mercaptoethylamine hydrochloride, cysteine, mercaptoethanol, and sodium bisulfite. The 2-mercaptopyrimidine adduct could not be obtained directly but was prepared from dibutyl mercaptoethaneboronate and 2-chloropyrimidine. The boronic acids have been tested in C_3H mice with subcutaneously implanted brain tumors to determine the ratio of boron in the tumor to that in brain, blood, and muscle, as a function of time. One of the more favorable compounds on this basis was S-boronoethylcysteine. High transient boron ratios were found to be inadequate, and the need for binding compounds to tumor with concomitantly low boron concentrations in blood and brain is discussed.

The possibility of treating intracranial tumors using neutron capture irradiation was first proposed by Sweet and Javid.[2] This type of therapy utilizes the fact that while thermal neutrons have insufficient energy to ionize tissue components, their absorption by certain nonradioactive nuclides with a high propensity for these neutrons, such as the boron isotope B^{10}, results in a fission reaction in which the emitted fragments possess considerable ionizing energy. In the case of B^{10} the following nuclear reaction occurs.

$$B^{10} + n^1 \rightarrow [B^{11}] \rightarrow Li^7 + He^4 + 2.4 \text{ Mev.}$$

These two particles are of such size that they travel a maximum of 9 μ, thereby releasing this destructive energy only in the immediate vicinity of the site of the original B^{10} atom. The main problem in the successful utilization of this technique has been the attainment of high levels of boron in the neoplasm with concomitantly low concentrations in normal tissue surrounding this area and in areas which will be subjected to thermal neutron bombardment.

Evaluation of a variety of substituted benzeneboronic acids in C_3H mice with subcutaneously transplanted brain tumors had shown that the most favorable tumor–brain boron ratios, as well as compounds with low toxicity, were found among those boronic acids having a high water–lipid solvent partition coefficient.[3a,b] Thus a low lipid solubility seemed to be an important requirement for a boron compound. As clinical information became available, it was apparent that the problem was more involved than the mere attainment of a high tumor–brain boron ratio. However, the preparation and evaluation of a series of highly water-soluble boronic acids was chosen as the goal of the present work on the basis of these first results.

Synthetic methods have not been available previously for the general preparation of alkylboronic acids containing hydrophilic substituents. The facile radical-catalyzed addition of mercaptans to the double bond of dibutyl ethyleneboronate[4] has been exploited in this present work to synthesize several boronic acids containing other functional groups, many of which have imparted increased water solubility.

The additions of mercaptoacetic and β-mercaptopropionic acid to dibutyl ethyleneboronate were easily accomplished. Either ultraviolet light or azobisisobutyronitrile could be used to initiate the reaction, but with additional experience it appeared that the azonitrile gave more consistent and better controlled results. The initially formed liquid adducts decomposed on attempted distillation and were therefore hydrolyzed directly to the corresponding boronic acids, 2-boronoethylthioacetic (**1a**), and 3-(2-borono-

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