for the secondary amines considered alone. Best correlations were found with log CMC.

Notable results were obtained for the tertiary amines. Fair correlations were found with log CMC (linear and quadratic) but not log P or E_s. However, very significant improvements were obtained when E_s was used together with either log CMC or log P. The clear implication is that for tertiary amines the bulk of the head group must be considered along with the overall hydrophobic properties of the molecule and has a deactivating effect. Somewhat similar results were found for a series of alcohols (15). Hansch and Glave (5) also noted structure–activity similarities for long chain amines and alcohols. They proposed that both classes of compounds be classified as "membrane-perturbing" agents.

The dodecylamines, like the secondary amines, yielded no significant correlations. This finding underscores the inability of the physicochemical parameters studied to represent adequately the biologically important properties of the ammonium head. However, the range of activities for this series is rather narrow.

In summary, the activity is dominated by the length of the long aliphatic tail. Best correlations are found with physicochemical properties sensitive to this feature. Substitution of small alkyl groups on the amine nitrogen has only a secondary effect on activity. For secondary amines, no rationale for the direction or magnitude of this effect was uncovered. For tertiary amines, the bulk of the ammonium head appears to be important when considered with hydrophobic properties. CMC was superior to the partition coefficient for quantitative structure–activity correlations.

REFERENCES

(1) G. Domagk, *Deut. Med. Wochenschr.*, **61**, 829(1935); through *Chem. Abstr.*, **29**, 7018(1935).

(2) A. T. Fuller, *Biochem. J.*, **36**, 548(1942).

(3) N. D. Weiner, F. Hart, and G. Zografi, *J. Pharm. Pharmacol.*, **17**, 350(1965).

(4) E. J. Lien, C. Hansch, and S. M. Anderson, *J. Med. Chem.*, **11**, 430(1968).

(5) C. Hansch and W. R. Glave, *Mol. Pharmacol.*, **7**, 337(1971).

(6) C. Hansch and E. J. Lein, *J. Med. Chem.*, **14**, 653(1971).

(7) J. J. Kabara, A. J. Conley, and J. P. Truant, *Antimicrob. Ag. Chemother.*, **2**, 492(1972).

(8) J. Ferguson, *Proc. Roy. Soc., Ser. B*, **127**, 387(1939).

(9) J. Iwasa, T. Fujita, and C. Hansch, *J. Med. Chem.*, **8**, 150(1965).

(10) C. Hansch, J. E. Quinlan, and G. L. Lawrence, *J. Org. Chem.*, **33**, 347(1968).

(11) A. W. Adamson, "Physical Chemistry of Surfaces," 2nd ed., Interscience, New York, N.Y., 1967, pp. 24–491.

(12) L. J. Powers, E. O. Dillingham, and G. E. Bass, *J. Pharm. Sci.*, **64**, 883(1975).

(13) G. Del Re, in "Electronic Aspects of Biochemistry," B. Pullman, Ed., Academic, New York, N.Y., 1964, p. 221. G. Del Re, *J. Chem. Soc.*, **1958**, 4031.

(14) W. P. Purcell, G. E. Bass, and J. M. Clayton, "Strategy of Drug Design," Wiley, New York, N.Y., 1973, pp. 69–71.

(15) E. O. Dillingham, R. W. Mast, G. E. Bass, and J. Autian, *J. Pharm. Sci.*, **62**, 22(1973).

ACKNOWLEDGMENTS AND ADDRESSES

Received September 9, 1974, from the *Department of Molecular Biology, College of Pharmacy, University of Tennessee Center for the Health Sciences, Memphis, TN 38163*

Accepted for publication February 11, 1975.

Supported in part by U.S. Public Health Service Grants DE-03429 and HL-09495.

The authors gratefully acknowledge the technical assistance of J. N. Warren and I. Bilsky.

[x] To whom inquiries should be directed.

5-Aryloxy-6-methoxy-8-aminoquinolines as Potential Prophylactic Antimalarials

KALIDAS PAUL and C. DeWITT BLANTON, Jr. [x]

Abstract □ 5-(*p*-Anisyloxy)-6-methoxy-8-(5-isopropylaminopentylamino)quinoline was resynthesized for evaluation in the *Plasmodium berghei* and monkey prophylactic (*Plasmodium cynomolgi*) tests. A new primary amine, three secondary amines, and one structurally modified side-chain analog of the 5-aryloxy series were also prepared. None of these compounds showed significant antimalarial or prophylactic activity.

Keyphrases □ 8-Aminoquinolines, 5- and 6-substituted—series synthesized, screened for antimalarial activity □ Antimalarial agents, potential—5- and 6-substituted 8-aminoquinolines synthesized and screened □ Structure–activity relationships—5- and 6-substituted 8-aminoquinolines synthesized and screened for antimalarial activity

During studies (1, 2) on the synthesis and evaluation of various derivatives of the 8-aminoquinoline nucleus as potential prophylactic antimalarials, a known compound, 5-(*p*-anisyloxy)-6-methoxy-8-(5-isopropylaminopentylamino)quinoline (I) (3, 4), was proposed for resynthesis and evaluation in the *Plasmodium berghei* and monkey prophylactic (*Plasmodium cynomolgi*) tests. In *Plasmodium gallinaceum* studies (4), I was relatively nontoxic and highly active, displaying the highest therapeutic index (177) of any 8-aminoquinoline tested. It was suggested that I and related compounds may be more conveniently converted to the *o*-quinoid structure *in vivo*, a structural feature proposed for the active metabolite (5, 6). Primaquine and pentaquine exhibited therapeutic indexes of 30 and 57, respectively, in this test (4).

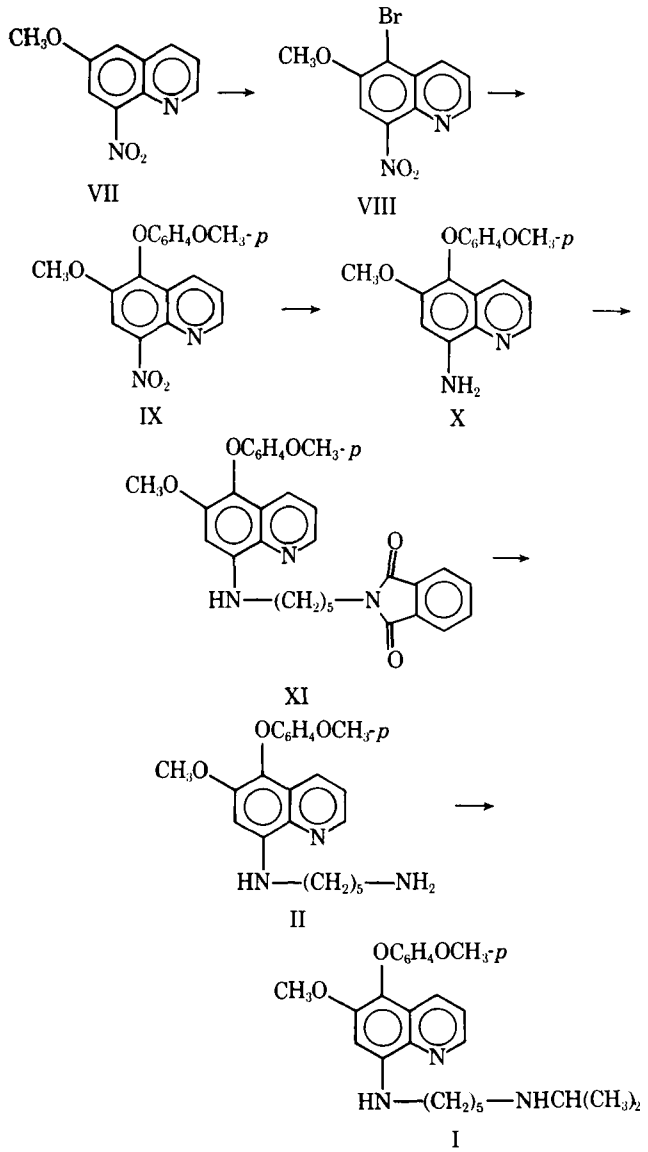
Although the 8-aminoquinolines are quite toxic, primaquine has had a prominent place in malaria prophylaxis. The possibility that 5-aryloxy-substituted 8-aminoquinolines may offer a lead in the search for more effective and less toxic agents led to a reexamination of I as well as a few selective, structurally modified analogs (II–VI). For example, the 5-aryloxy analog (II) of primaquine was considered since primaquine has been recognized as the least toxic and most effective 8-aminoquinoline tested in humans (5, 7). It was reported (7, 8) that occasional aromatic interruption in

the aminoalkylamino side chain provides relatively nontoxic compounds with a high therapeutic index. Compound VI has incorporated this structural feature.

DISCUSSION

The approach for the synthesis of I is a modification of the original literature procedure (3). In this modification (Scheme I), the new primary amine (II) (Table I) was obtained as an intermediate during the preparation of the known agent, I. Compound II served as the key intermediate for the preparation of additional analogs (III–V). The original procedure (3) called for nucleophilic displacement of the 5-bromo atom of VIII by potassium *p*-methoxyphenoxide, which was generated *in situ*. This method gave inconsistent results (10–30% yields). However, if the potassium *p*-methoxyphenoxide is prepared and isolated, the dried salt reacts with VIII, in dimethylformamide, to give yields consistently greater than 70%. A second modification employed 5-bromo-1-phthalimidopentane instead of the 1-halo-5-(isopropylamino)pentane of the original method (3).

Although elemental analyses for the key intermediate (XI) employed to synthesize I and II gave a consistently low value for carbon when it was not considered as a hemihydrate, the product obtained by Methods A and B gave identical IR and NMR spectra. TLC results were homogeneous and identical. Preparation of VI employed a similar process, utilizing 1,4-bis(chloromethyl)benzene for the synthesis of the intermediate, 4-phthalimidomethylbenzyl chloride (XII), required for reaction with X.



Scheme I

BIOLOGICAL ACTIVITY

The antimalarial activity[1] was assessed against *P. berghei* in mice by the method of Osdene *et al.* (9) (Table II). With this test, only IIa met the criteria to be considered "active." Furthermore, I, which was such a promising candidate in the *P. gallinaceum* test (4), was a failure in terms of activity and toxicity when compared to primaquine in the *P. berghei* test.

Compounds I, II, and VI also were evaluated in monkeys for prophylactic and radical curative activity by the Schmidt technique (10, 11). This method involves sporozoite-induced *P. cynomolgi* infections of rhesus monkeys. All compounds were inactive at 10 mg/kg. Primaquine was active at 1 mg/kg. The activity patterns did not justify expanded testing or further extension of the present group.

EXPERIMENTAL[2]

5-Bromo-6-methoxy-8-nitroquinoline (VIII)—Compound VIII was prepared in 86% yield according to the procedure of Elderfield *et al.* (12), mp 207–209° [lit. (12) mp 203–205.5°].

5-(*p*-Anisyloxy)-6-methoxy-8-nitroquinoline (IX)—Preparation of this compound by the existing procedure (3) gave irreproducible yields, varying from 10 to 30%. The literature procedure for reaction of VIII and potassium *p*-methoxyphenoxide was modified to give yields as high as 70%. The modifications included a change in the reaction solvent from butyl cellulose to dimethylformamide and isolation of the potassium *p*-methoxyphenoxide, instead of generating *in situ*, and drying.

5-(*p*-Anisyloxy)-6-methoxy-8-aminoquinoline (X)—Compound X was prepared in 55–90% yield according to the procedure of Lauer *et al.* (13), mp 112–115° [lit. (13) mp 115–116°].

5 - (*p*-Anisyloxy)-6-methoxy-8-(5-phthalimidopentylamino)quinoline (XI)—*Method A*—An intimate mixture of 11.84 g (40 mmoles) of X and 5.94 g (20 mmoles) of 5-bromophthalimidopentane was heated at 155–160° for 3 hr (under nitrogen). The resulting black mass was extracted with 1600 ml of benzene (200-ml portions). The extract was treated with charcoal, filtered, and concentrated to give 3 g of canary yellow solid, mp 150–152° (after recrystallization from ethanol–chloroform, mp 152–153°).

Anal.—Calc. for $C_{30}H_{29}N_3O_5$: C, 70.43; H, 5.71; N, 8.21. Calc. for $C_{30}H_{29}N_3O_5 \cdot 0.5H_2O$: C, 69.22; H, 5.80; N, 8.07. Found: C, 69.14, 69.06; H, 5.91, 5.86; N, 8.06.

Method B—5-Bromophthalimidopentane (6 g) and 6 g of X in 100 ml of 66% ethanol and 136 g of sodium acetate were refluxed for 72 hr. After 24 hr, 6 g of 5-bromophthalimidopentane and 13.6 g of sodium acetate were added; after 48 hr, 27.2 g of sodium acetate was added to keep the pH at 7–8. The reaction mixture was cooled, saturated with potassium carbonate, and extracted with ether (some canary yellow precipitate appeared at the interphase). This solid was taken with the ether layer and filtered to give 5 g (50% yield). Evaporation of the dried ether layer gave a dark oil, which was mainly unreacted amine (X) and 5-bromophthalimidopentane. The analytical material obtained from ethanol–chloroform melted at 152–153°.

Anal.—Calc. for $C_{30}H_{29}N_3O_5$: N, 8.21. Found: N, 8.08.

5-(*p*-Anisyloxy)-6-methoxy-8-(5-aminopentylamino)quinoline Dimaleate (IIa)—Compound XI (39 g, 0.0763 mole) was suspended in 400 ml of 95% ethanol and 35 ml of 85% hydrazine hydrate. The suspension was heated in an oil bath (90–100°) for 1 hr to give a clear solution, followed by the appearance of a voluminous white precipitate. After heating for an additional 1 hr, a portion of the ethanol was removed *in vacuo*. The solid residue was cooled to room temperature and dissolved in 45 ml of water containing 45 g of sodium

[1] Antimalarial test results were provided by the Walter Reed Army Institute of Research.

[2] Melting points were determined on a Thomas-Hoover apparatus (capillary method) and are uncorrected. NMR spectra were determined on a Hitachi Perkin-Elmer R 20A high-resolution NMR spectrometer using tetramethylsilane as the internal reference. IR spectra were determined on a Perkin-Elmer 237B grating spectrophotometer using the potassium bromide technique. Elemental analyses were determined by Atlantic Microlab, Inc., Atlanta, Ga. TLC was performed on Eastman Chromatogram sheets, type 6060 (silica gel).

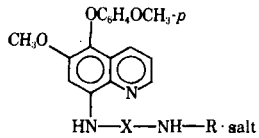
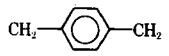


Table I—5-Aryloxy-6-methoxy-8-aminoquinolines

Compound	X	R	Salt	Yield, %	Melting Point	Formula	Analysis, % Calc.	Analysis, % Found
I	$(CH_2)_5$	$CH(CH_3)_2$	Maleate	84.0	120–122°	$C_{29}H_{37}N_3O_7$	C 64.54 H 6.91 N 7.78	64.66 6.94 7.72
IIa	$(CH_2)_5$	H	Dimaleate	68.4	118–120°	$C_{30}H_{35}N_3O_{11}$	C 58.72 H 5.74 N 6.84	58.54 6.05 6.83
IIb	$(CH_2)_5$	H	Maleate	—	127–128°	$C_{26}H_{31}N_3O_7$	C 62.76 H 6.28 N 8.45	62.92 6.38 8.36
IIc	$(CH_2)_5$	H	—	—	61–65[a]	$C_{22}H_{27}N_3O_3$	—	—
III	$(CH_2)_5$	2-Adamantyl	Maleate	87.0	153–155°	$C_{36}H_{45}N_3O_7$	C 68.44 H 7.17 N 6.65	68.21 7.28 6.62
IV	$(CH_2)_5$	3,4,5-Trimethoxybenzyl	Tartrate	51.5	119–121°	$C_{37}H_{45}N_3O_{12}\cdot H_2O$	C 59.90 H 6.34 N 5.66	59.83 6.53 5.68
V	$(CH_2)_5$	2-Hydroxybenzyl	Tartrate	96.0	90–95°	$C_{33}H_{39}N_3O_{10}$	C 62.16 H 6.16 N 6.58	61.93 6.20 6.54
VI		H	Maleate	77.5	158–160°	$C_{29}H_{29}N_3O_7$	C 65.53 H 5.50 N 7.90	65.23 5.59 7.86

hydroxide. This solution was treated with 300 ml of ether, stirred at room temperature for 1 hr, and allowed to stand overnight at room temperature.

The ether layer was separated, washed with water and a saturated salt solution, and dried over anhydrous sodium sulfate. Concentration of the ether gave an oil (32 g). This oil was redissolved in 300 ml of ether and treated with 500 ml of a saturated solution of maleic acid in ether (20 g of maleic acid/500 ml of ether). A red precipitate (32 g, 68.4%) was obtained, mp 120–122°. The analytical sample was prepared by recrystallizing from chloroform, mp 118–120° (see Table I for physical constants).

By continuous recrystallization, it was possible to obtain the monomaleate (IIb), mp 127–128°. Suspension of IIa or IIb in 1 *N* sodium hydroxide gave a yellow solid, mp 61–65°. The free base (IIc) was not analyzed but was used directly for the synthesis of III–V. These compounds gave expected NMR and IR spectra.

5-(*p*-Anisyloxy)-6-methoxy-8-(5-isopropylaminopentylamino)quinoline Maleate (I)—Compound IIc (5.7 g, 15 mmoles), 3 ml of acetone, 0.5 g of prereduced platinum oxide, and 75 ml of absolute ethanol were shaken at room temperature on a Parr hydrogenation apparatus (initial pressure of 50 psi). After 5 hr, 0.6 kg (1.3 lb) of pressure had been lost. The catalyst was removed, and the ethanol solution was concentrated *in vacuo*.

The resulting oil was dissolved in ether and dried over anhydrous sodium sulfate. The ether was removed *in vacuo*, and the oil was redissolved in anhydrous ether. This solution was treated with a saturated ether solution of maleic acid to give a red precipitate (6.8 g, 84%), mp 110–112°. The maleate salt was dissolved in chloroform, treated with charcoal, and precipitated by addition of ether as a yellow solid (5.5 g), mp 120–122°.

5 - (*p*-Anisyloxy)-6-methoxy-8-[5-(3,4,5-trimethoxybenzyl)-aminopentylamino]quinoline *d*-Tartrate (IV)—Compound IIc (1.2 g, 315 mmoles), 0.8 g of 3,4,5-trimethoxybenzaldehyde, 50 ml of benzene, and 2–3 drops of piperidine were refluxed, with azeotropic removal of water, for 18 hr. On removal of the benzene, an oil was obtained; this oil gave a yellow solid (mp 65–70°) on trituration with methanol (1.9 g). Without further purification, this yellow solid (Schiff base) was dissolved in 50 ml of absolute ethanol, warmed to 50°, and treated with 2 g of sodium borohydride.

After the addition was completed, the solution was heated at 60° for 0.5 hr, cooled to room temperature, and diluted with water. The aqueous solution was extracted with ether, the ether layer was dried over sodium sulfate, and the product was isolated as a *d*-tartrate salt. The analytical sample was obtained by dissolving in chloroform and reprecipitating (1.2 g, 51.5%) with ether, mp 119–121°.

5 - (*p*-Anisyloxy)-6-methoxy-8-[5-(2-adamantyl)aminopentylamino]quinoline Maleate (III)—This compound was prepared from 2-adamanone and the free base (IIc) in 87% yield by the procedure already outlined, mp 153–155°.

5-(*p*-Anisyloxy)-6- methoxy-8-[5- (2-hydroxybenzyl)amino-

Table II—Antimalarial Activity against *P. berghei*[a]

Compound	ΔMST (days) after a Single Dose 20 mg/kg sc	40 mg/kg sc	80 mg/kg sc	160 mg/kg sc	320 mg/kg sc	640 mg/kg sc
I	—	0.3	—	0.9 (2T)	—	(5T)
IIa	1.1	1.7	3.1	4.7	5.7	7.3
IIb	0.7	0.9	2.1	4.3	4.7	5.7
IIc	0.5	1.3	3.1	3.5	4.5	5.9
III	0.3	0.5	1.5	2.5	3.3	5.7
IV	—	0.1	—	0.1	—	0.1
V	—	0.3	—	0.5	—	0.5
VI	—	0.3	—	0.5	—	0.5
Primaquine phosphate	4.0	5.0	9.4	10.8 (2T)	(5T)	(5T)

[a] Increase in mean survival time (MST), in days, of the test group (five mice) is reported. The mean survival time of untreated mice is 6.1 days. A compound is "active" if ΔMST exceeds 6.1 days. Animals that survive to 60 days postinfection are considered "cured" (C). Deaths from Days 2–5 after drug administration are attributed to drug toxicity (T).

pentylamino]quinoline *d*-Tartrate (V)—This compound was prepared from salicylaldehyde by the procedure previously described. A yield of 96% was obtained, mp 90–95° (with bubbling at 80°).

4-Phthalimidomethylbenzyl Chloride (XII)—1,4-Bis(chloromethyl)benzene (43.7 g, 0.25 mole) was added to 300 ml of dimethylformamide. Potassium phthalimide (46 g, 0.25 mole) was added in two portions. The second portion was added after 3 hr together with another 300 ml of dimethylformamide. The mixture was heated for 3 hr at 85°, and 500 ml of dimethylformamide distilled off under aspirator vacuum (about 18–19 hr). The residue was poured into water, and a white material was collected. This material was extracted with 1 liter of acetone, leaving 18.5 g of the insoluble diphthalimido product. Extraction of the acetone solution with hot petroleum ether (bp 30–60°) removed 12 g of unreacted 1,4-bis(chloromethyl)benzene. From the acetone solution was obtained 33.5 g of desired product, mp 143–146°.

5 - (*p*-Anisyloxy)-6-methoxy-8-(4-phthalimidomethylbenzylamino)quinoline (XIII)—Compound XII (2.8 g), 1.6 g of potassium iodide, and 20 ml of acetone were boiled for 1 hr and concentrated. The aminoquinoline (X) (3 g) was added together with 1.3 g of anhydrous potassium carbonate and 20 ml of 2-propanol. This mixture was heated in an open flask on a steam bath for 1.5 hr, allowing the 2-propanol to evaporate. The residue was poured into water, made basic with potassium carbonate, extracted with chloroform, and dried (sodium sulfate).

The chloroform was removed *in vacuo*, and the residue was heated with absolute ethanol to give an orange solid. This solid was dissolved in benzene, treated with charcoal, filtered, and concentrated to an oily residue. Addition of warm absolute ethanol gave an orange solid, which was collected and washed with absolute ethanol, mp 164–165°. The yield was 3 g (55%). In a second experiment, the yield was increased to 76%.

Anal.—Calc. for $C_{33}H_{27}N_3O_5$: C, 72.65; H, 4.99; N, 7.70. Found: C, 72.82; H, 5.04; N, 7.58.

5-(*p*-Anisyloxy)-6-methoxy-8-(4-aminomethylbenzylamino)-quinoline Maleate (VI)—The phthalimido compound (XIII) (11 g, 0.02 mole) in 200 ml of 95% ethanol and 10 ml of 85% hydrazine hydrate was refluxed for 1.5 hr at 90–100°. Ethanol was removed, 20 g of potassium hydroxide in 20 ml of water was added, and the mixture was stirred for 0.5 hr. The solution was extracted with ether (500 ml), dried over sodium sulfate, concentrated, and redissolved in ether. A saturated ether solution of maleic acid (11 g/500 ml of ether) was added. The yellowish orange precipitate (hygroscopic) was filtered, dissolved in chloroform, and reprecipitated with ether, mp 136–139°. It analyzed as the dimaleate salt.

Anal.—Calc. for $C_{25}H_{25}N_3O_3 \cdot (C_4H_4O_4)_2$: C, 61.20; H, 5.16; N, 6.49. Found: C, 60.91; H, 5.24; N, 6.72.

Two additional reprecipitations from chloroform by ether gave the product as the monomaleate salt (77.5%), mp 158–160° (Table I).

REFERENCES

(1) K. Paul and C. D. Blanton, Jr., *J. Med. Chem.*, **16**, 1391(1973).

(2) W. P. Wetter and C. D. Blanton, Jr., *ibid.*, **17**, 620(1974).

(3) N. L. Drake, R. A. Hayes, J. A. Garman, R. B. Johnston, G. W. Kelley, S. Melamed, and R. M. Peck, *J. Am. Chem. Soc.*, **71**, 455(1949).

(4) G. R. Coatney, W. C. Cooper, N. B. Eddy, and J. Greenberg, "Survey of Antimalarial Agents," Public Health Monograph No. 9, U.S. Government Printing Office, Washington, D.C., 1953, p. 47.

(5) P. B. Russell, in "Medicinal Chemistry," 2nd ed., A. Burger, Ed., Interscience, New York, N.Y., 1960, p. 814.

(6) F. Schonhofer, *Z. Physiol. Chem.*, **274**, 1(1942).

(7) P. E. Thompson and L. M. Werbel, "Antimalarial Agents," Academic, New York, N.Y., 1972, p. 100.

(8) A. Funke, D. Bovet, and G. Montezin, *Ann. Inst. Pasteur, Paris*, **72**, 264(1946).

(9) T. S. Osdene, P. B. Russell, and L. Rane, *J. Med. Chem.*, **10**, 431(1967).

(10) "Chemotherapy of Malaria and Resistance to Antimalarials," *WHO Tech. Rep. Ser.*, No. 529 (1973).

(11) "WHO Report of Procedures for Screening Potential Antimalarial Compounds," in World Health Organization (1972b); WHO/MAL/72.763 (cyclostyled report), WHO, Geneva.

(12) R. C. Elderfield, W. R. Vaughan, B. R. Millward, and J. H. Ross, *J. Org. Chem.*, **23**, 1378(1958).

(13) W. M. Lauer, C. Rondestvedt, R. T. Arnold, N. L. Drake, J. VanHook, and J. Tinker, *J. Am. Chem. Soc.*, **68**, 1546(1946).

ACKNOWLEDGMENTS AND ADDRESSES

Received September 8, 1975, from the *Department of Medicinal Chemistry, School of Pharmacy, University of Georgia, Athens, GA 30602*

Accepted for publication December 1, 1975.

Supported by the U.S. Army Medical and Development Command under Contract DADA 17-71-C1068.

This paper is Contribution No. 1375 from the Army Research Program on Malaria.

The authors thank Dr. R. E. Strube, Dr. E. A. Steck, and Dr. T. R. Sweeney of the Walter Reed Army Institute of Research for interest and encouragement.

[x] To whom inquiries should be directed.

Determination of Salicylates by GLC

JOHN P. TISCHIO

Abstract □ Silylation of salicylic acid by hexamethyldisilazane, *N,O*-bis(trimethylsilyl)acetamide, and *N,N*-bis(trimethylsilyl)trifluoroacetamide was compared using GLC. The completeness of the reaction, the stability of products, and the reproducibility of results with time were investigated. Different reaction vessels were examined for their reliability and application for a routine assay procedure.

Keyphrases □ GLC—analysis, salicylic acid, different silylating reagents and different reaction vessels compared □ Salicylic acid—GLC analysis, different silylating reagents and different reaction vessels compared □ Silylating reagents, various—compared in GLC analysis of salicylic acid □ Keratolytic agents—salicylic acid, GLC analysis

Several methods for the determination of salicylates in physiological fluids were reviewed previously (1). One of these, GLC, is attractive because it affords a sensitive quantitative method for the simultaneous determination of aspirin and salicylic acid. Several papers have reported different GLC procedures (1–5), but the most promising incorporate a silylation process after extraction of the salicylates from the physiological fluid. The silylating reagents that have been utilized include hexamethyldisilazane (2), *N,O*-bis(trimethylsilyl)-acetamide (3), and *N,N*-bis(trimethylsilyl)trifluoroacetamide (4).