

Figure 3—Correlation between HPLC and GLC-mass spectral methods for plasma imipramine and desipramine analysis. For desipramine, the slope = 1.01, the intercept = 4.7, and the correlation coefficient = 0.943. For imipramine, the slope = 1.55, the intercept = -7.2, and the correlation coefficient = 0.960.

tenance dose. Figure 2b shows a chromatogram of a plasma standard containing 50 ng/ml of each drug and metabolite and added internal standard. One milliliter each of the plasma standard and patient sample were extracted, and one-half of the extract was injected according to the procedure.

Eighteen plasma specimens from seven patients participating in an NIMH study (5) and receiving imipramine or desipramine were assayed by the HPLC method and a GLC-mass spectrometric method (18) (Fig. 3). The two assays showed excellent correlation, with a slope of nearly unity (1.01) for desipramine. The HPLC assay yielded higher results than the GLC-mass spectral method for imipramine. Imipramine and desipramine are stable at least 3 months in plasma stored at -4° . The NIMH samples were 6–15 months old at the time of HPLC analysis; therefore, interference in the fluorescence assay due to some unidentified degradation component(s) may explain the discrepancy between the methods for imipramine. Further comparison studies with fresh patient samples and spiked plasma are in progress. Data for the 2-hydroxy metabolites quantitated by GLC-mass spectra were not available for comparison.

Chemistry of 8-Chloroberberine

JEROME L. MONIOT, TINA M. KRAVETZ,
ABD EL RAHMAN H. ABD EL RAHMAN, and MAURICE SHAMMA *

Received June 1, 1978, from the Department of Chemistry, Pennsylvania State University, University Park, PA 16802. Accepted for publication November 21, 1978.

Abstract □ 8-Chloroberberine (V), obtained by treatment of oxyberberine (I) with phosphorus oxychloride, is a reactive intermediate. Treatment with ammonia, methylamine, *n*-propylamine, aniline, and *p*-toluidine furnished the 8-berberinylidene derivatives IV and VII-X. Reaction of V with malononitrile, ethyl acetoacetate, and ethyl malonate anions yielded the 8-berberinylidene derivatives XII-XIV. Acid hydrolysis of XIV gave 8-berberinylacetic acid (XV) whose reduction provided 8-canadanylacetic acid (XVI). Grignard reagents react readily with V. Methylmagnesium iodide, ethylmagnesium iodide, and benzylmagnesium iodide led to 8,8-dimethyldihydroberberine (XVII), 8,8-di-

- ### REFERENCES
- (1) L. F. Gram, *Clin. Pharmacokinet.*, **2**, 237 (1977).
 - (2) J. Christiansen and L. F. Gram, *J. Pharm. Pharmacol.*, **25**, 604 (1973).
 - (3) A. H. Glassman, J. M. Perel, M. Shostak, S. J. Kantor, and J. L. Fleiss, *Arch. Gen. Psychiat.*, **34**, 197 (1977).
 - (4) S. B. Jandhyala, M. L. Steenberg, J. M. Perel, A. A. Manian, and J. P. Buckley, *Eur. J. Pharmacol.*, **42**, 403 (1977).
 - (5) W. Z. Potter, A. P. Zavadil, and F. K. Goodwin, *Psychopharmacol. Bull.*, **14**, 29 (1978).
 - (6) W. Z. Potter, H. M. Calil, A. A. Manian, A. P. Zavadil, and F. K. Goodwin, in press.
 - (7) R. E. Heikkila, S. S. Goldfinger, and H. Orlansky, *Res. Commun. Chem. Pathol. Pharmacol.*, **13**, 237 (1976).
 - (8) D. C. Fenimore, C. J. Meyer, and C. M. Davis, *J. Chromatogr.*, **142**, 399 (1977).
 - (9) P. N. Kaul, L. R. Whitfield, and M. L. Clark, *J. Pharm. Sci.*, **67**, 60 (1978).
 - (10) A. Nagy and L. Treiber, *J. Pharm. Pharmacol.*, **25**, 599 (1973).
 - (11) D. N. Bailey and P. I. Jatlow, *Clin. Chem.*, **22**, 1697 (1976).
 - (12) R. G. Jenkins and R. O. Friedel, *J. Pharm. Sci.*, **67**, 17 (1978).
 - (13) J. C. Kraak and P. Bijster, *J. Chromatogr.*, **143**, 499 (1977).
 - (14) F. L. Vandemark, R. F. Adams, and G. J. Schmidt, *Clin. Chem.*, **24**, 87 (1978).
 - (15) J. H. M. Van Den Berg, H. J. J. M. DeRuwe, and R. S. Deelder, *J. Chromatogr.*, **138**, 431 (1977).
 - (16) I. D. Watson and M. J. Stewart, *ibid.*, **132**, 155 (1977).
 - (17) L. F. Gram, *Dan. Med. Bull.*, **21**, 218 (1974).
 - (18) M. Claeys, G. Muscettola, and S. P. Markey, *Biomed. Mass Spectrom.*, **3**, 110 (1976).

ACKNOWLEDGMENTS

Supported by Grant 20852 from the National Institutes of General Medical Sciences, National Institutes of Health.

The provision of GLC-mass spectral assay samples and GLC-mass spectral results by Dr. William Z. Potter of the National Institute of Mental Health and Dr. Anthony P. Zavadil, III, of the National Institutes of General Medical Sciences, the gifts of drug metabolites by Dr. Albert A. Manian of the National Institute of Mental Health, and the clinical assistance of Dr. Richard E. Wolin are appreciated.

Protoberberine salts and tetrahydroprotoberberine bases have wide pharmacological potency including tranquilizing, antimicrobial, antifungal, hypotensive, antiulcer, uterine, antiarrhythmic, neuroleptic, and antipsychotic

effects. Protoberberine salt pharmacology was reviewed recently (1, 2).

The present report describes the structure of 8-chloroberberine (V), a compound characterized partially by

previous researchers (3). 8-Chloroberberine was then used as an intermediate in the preparation of new berberine derivatives.

DISCUSSION

Initial investigations of phosphorus oxychloride-oxyberberine (I) reaction products identified oxyberberine (II) and a reactive chlorinated derivative, tentatively assigned Structure III (3). Treatment of the chlorinated derivative with ammonia yielded iminoberberine (IV), characterized as its hydrochloride salt. When iminoberberine was subjected to aqueous alkali, hydrolysis to I took place (3).

Refluxing I with phosphorus oxychloride produced the stable but moisture-sensitive orange crystalline salt 8-chloroberberine (V), which readily hydrolyzed to I in water, ethanol, or aqueous alkali. The V high-resolution mass spectrum contained the molecular ion m/e 370 for $C_{20}H_{17}NO_4Cl$. The berberine and V trifluoroacetic acid PMR spectra were nearly identical, the major difference being the absence in V of the proton singlet at δ 10.1 assigned to H-8 in the spectrum of berberine chloride. Thus, the phosphorus oxychloride product from I is the C-8 monochloro berberine analog V rather than the dichloro compound III. The high V reactivity toward oxygen nucleophiles was extended to other entering groups including primary amines, carbanions, and Grignard reagents.

Reaction of V with anhydrous ammonia produced Perkin's yellow "iminoberberine" (IV), here designated 8-berberinylideneimine. Acetylation of this material produced the yellow *N*-acetyl amide VI. Mild VI hydrolysis with sodium bicarbonate regenerated IV.

Similarly, V reacted with methylamine, *n*-propylamine, aniline, and *p*-toluidine to give the 8-berberinylidene derivatives VII-X. As partial verification of these results, IV methylation with methyl iodide produced 8-berberinylidenemethylimine (VII), identical to that obtained by direct treatment of V with methylamine.

Imine bases VII-X hydrolysis with sodium hydroxide regenerated I while reduction of the bases with sodium borohydride in ethanol supplied canadine (XI) (4).

Since V reacts readily with basic nitrogen nucleophiles, V reaction with carbanions was also investigated. Treatment of V with the malononitrile anion furnished the dark-orange 8-berberinylidenemalononitrile (XII). Reaction of V with ethyl acetoacetate and ethyl malonate anions yielded the 8-berberinylidene derivatives XIII and XIV, respectively. Acid hydrolysis of XIV was accompanied by decarboxylation and produced the orange 8-berberinylacetic acid (XV). Reduction of this quaternary salt with sodium borohydride in ethanol generated 8-canadinylacetic acid (XVI).

Another aspect of V chemistry is its reactivity toward Grignard reagents. With methylmagnesium iodide, a good yield of 8,8-dimethyldihydroberberine (XVII) was obtained; with ethylmagnesium iodide, 8,8-diethyldihydroberberine (XVIII) was produced. The latter compound was reduced with sodium borohydride to 8,8-diethylcanadine (XIX). Monoalkylation of V with the bulkier benzylmagnesium bromide gave the benzyl derivative XX whose borohydride reduction product was identical with 8-benzylcanadine (XXI) (5).

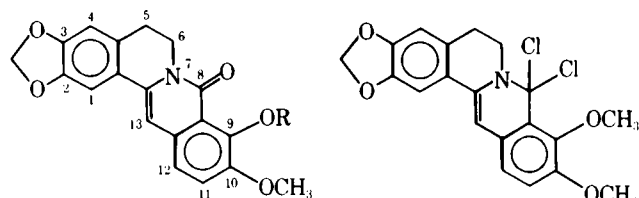
Clearly, the readily available V is a useful synthetic intermediate for the generation of 8-substituted berberine and canadine derivatives.

EXPERIMENTAL¹

8-Chloroberberine (V)—An oxyberberine (I) slurry (5 g, 14.2 mmoles) in phosphorus oxychloride (10 ml) was refluxed for 2 hr. After cooling, the orange-red crystalline residue was collected, washed with chloroform and then ether, and dried *in vacuo* to yield 4.5 g (85%) of V as orange-red crystals, mp >300°; PMR (trifluoroacetic acid): δ 3.30 (t, 2H, $J = 7$ Hz, H-5), 4.23 (s, 6H, 2 OCH₃), 5.20 (t, 2H, $J = 7$ Hz, H-6), 6.13 (s, 2H, OCH₂O), 6.97 (s, 1H, H-4), 7.47 (s, 1H, H-1), 8.10 (s, 2H, H-11, 12), and 8.47 (s, 1H, H-13); mass spectrum: m/e M⁺ 370 (10), 355 (10), and 335 (100); high-resolution mass spectrum: calc. for $C_{20}H_{17}ClNO_4$, 370.0845; found m/e 370.0876.

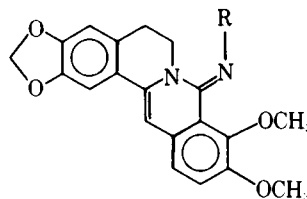
Compound V Hydrolysis—Compound V was decolorized rapidly with water, ethanol, or aqueous alkali and gave I exclusively.

8-Berberinylideneimine (IV)—Anhydrous ammonia gas was bub-

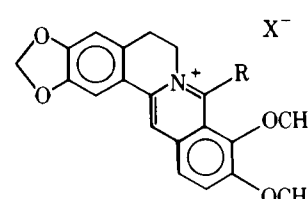


I: R = CH₃
II: R = H

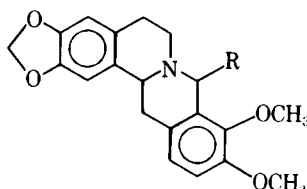
III



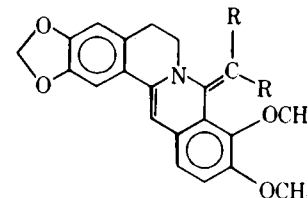
IV: R = H
VI: R = COCH₃
VII: R = CH₃
VIII: R = CH₃CH₂CH₂
IX: R = Ph
X: R = *p*-CH₃C₆H₅



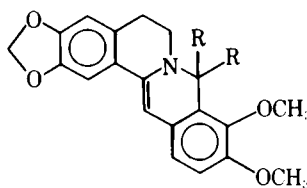
V: R = Cl
XV: R = CH₂COOH
XX: R = CH₂C₆H₅



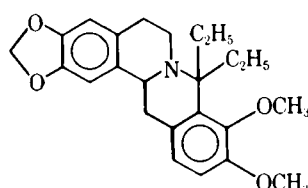
XI: R = H
XVI: R = CH₂COOH
XXI: R = CH₂C₆H₅



XII: R = CN
XIII: R = COOC₂H₅, COCH₃
XIV: R = COOC₂H₅



XVII: R = CH₃
XVIII: R = C₂H₅



XIX

bled through a V slurry (1 g, 2.7 mmoles) in benzene (20 ml) for 1 hr. Following solvent removal, the residue was washed with water and partitioned between chloroform and aqueous sodium bicarbonate. The organic layer was dried, and the solvent was evaporated to afford yellow needles (0.9 g, 95%), which were recrystallized from chloroform, mp >300° [lit. (2) mp >280°]; IR: $\nu_{max}^{CHCl_3}$ 1640 and 3300 cm⁻¹; UV: $\lambda_{max}^{ethanol}$ 282 (log ϵ 3.48), 358 (3.76), 377 sh (3.61), and 400 (3.41) nm; UV: $\lambda_{max}^{ethanol.H^+}$ 233 (log ϵ 4.05), 253 sh (3.77), 343 (3.83), 386 (3.48), and 403 sh (3.40) nm; PMR (CDCl₃): δ 2.87 (t, 2H, $J = 7$ Hz, H-5), 3.88 (s, 3H, OCH₃), 3.92 (s, 3H, OCH₃), 4.23 (t, 2H, H-6), 5.93 (s, 2H, OCH₂O), 6.12 (s, 1H, H-13), 6.63 (s, 1H, H-4), 7.05 (s, 2H, H-11, 12), and 7.13 (s, 1H, H-1); PMR (trifluoroacetic acid): δ 3.17 (t, 2H, $J = 7$ Hz, H-5), 4.14 (s, 3H, OCH₃), 4.27 (s, 3H, OCH₃), 4.35 (t, 2H, H-6), 6.10 (s, 2H, OCH₂O), 5.85 (br s, 3H, NH), 6.91 (s, 1H, H-4), 7.32 (s, 1H, H-1), 7.50 (s, 1H, H-13), and 7.80 (s, 2H, H-11, 12); mass spectrum: m/e M⁺ 350 (100), 335 (80), 321 (20), and 293 (50).

Sodium borohydride in ethanol reduction of IV provided (\pm)-canadine (XI), mp 172–173° [lit. (4) mp 170–171°], quantitatively.

The IV-HCl had a melting point of 165–166° (methanol).

Anal.—Calc. for $C_{20}H_{18}N_2O_4 \cdot HCl$: C, 62.24; H, 4.93. Found: C, 61.98; H, 5.05.

***N*-Acetyl-8-berberinylideneimine (VI)**—A slurry of IV (1 g, 2.8 mmoles) in pyridine (10 ml) and acetic anhydride (5 ml) was stirred overnight. After solvent removal, the residue crystallized from methanol as tan needles (0.84 g, 2.1 mmoles, 75%), mp 226–228°; IR: ν_{max}^{KBr} 1655 cm⁻¹; UV: $\lambda_{max}^{ethanol}$ 263 (log ϵ 4.22), 348 (4.23), 408 (3.68), and 420 (3.69) nm; PMR (trifluoroacetic acid): δ 2.70 (s, 3H, CH₃CO), 3.18 (t, 2H, $J = 7$ Hz, H-5),

¹ PMR spectra were obtained at 60 MHz in deuteriochloroform, unless specified otherwise, with tetramethylsilane as the internal standard. Mass spectra were run at 70 eV. TLC utilized Merck 254 silica gel plates. Microanalyses were done at Midwest Microlab, Indianapolis, Ind.

4.24 (s, 3H, OCH₃), 4.37 (s, 3H, OCH₃), 4.72 (t, 2H, *J* = 7 Hz, H-6), 6.18 (s, 2H, OCH₂O), 6.94 (s, 1H, ring A aromatic H), 7.49 (s, 1H, ring A aromatic H), 8.40 (s, 1H, H-13), and 8.08 (s, 2H, ring D aromatic H); mass spectrum: *m/e* M⁺ 392 (5), 377 (5), 361 (100), 352 (15), 335 (17), 320 (10), 305 (5), and 291 (25); high-resolution mass spectrum: calc. for C₂₂H₂₀N₂O₅, 392.1373; found *m/e* 392.1387.

Compound VI hydrolysis with aqueous sodium bicarbonate and chloroform extraction produced IV in near-quantitative yield.

Preparations of 8-Berberinylidenemethylamine (VII), 8-Berberinylidene-*n*-propylamine (VIII), 8-Berberinylideneaniline (IX), and 8-Berberinylidene-*p*-toluidine (X)—The anhydrous amine, in excess, was added to a stirred V benzene slurry, and the mixture was stirred for 1 hr. After dilution with ether, dry hydrogen chloride gas was added until precipitation was complete. The collected solid was washed with water and dissolved in chloroform, and the solution was dried. Solvent evaporation was followed by crystallization. The yields were around 80%.

Compound VII, C₂₁H₂₀N₂O₄, was obtained as light-yellow needles, mp 122° (methanol); IR: $\nu_{\text{max}}^{\text{CHCl}_3}$ 1570 and 1640 cm⁻¹; UV: $\lambda_{\text{max}}^{\text{ethanol}}$ 283 (log ϵ 3.83) and 360 (4.16) nm; UV: $\lambda_{\text{max}}^{\text{ethanol, H}^+}$ 253 (log ϵ 4.18), 340 (4.25), and 395 (3.78) nm; PMR (trifluoroacetic acid): δ 3.15 (t, 2H, *J* = 7, H-5), 3.51 (s, 3H, NCH₃), 4.18 (s, 3H, OCH₃), 4.30 (s, 3H, OCH₃), 4.54 (t, 2H, *J* = 7 Hz, H-6), 61.0 (s, 2H, OCH₂O), 6.91 (s, 1H, aromatic H), 7.32 (s, 1H, aromatic H), 7.50 (s, 1H, aromatic H), and 7.80 (s, 2H, H-11,12); mass spectrum: *m/e* M⁺ 364 (30), 350 (90), 335 (100), and 291 (10); high-resolution mass spectrum: calc. for C₂₁H₂₀N₂O₄, 364.1419; found *m/e* 364.1446.

The VII-HCl salt had a melting point of 187–189° (methanol).

Compound VIII, C₂₃H₂₄N₂O₄, was obtained as light-yellow needles, mp 170–172° (ether); UV: $\lambda_{\text{max}}^{\text{ethanol}}$ 283 (log ϵ 4.09) and 363 (4.22) nm; UV: $\lambda_{\text{max}}^{\text{ethanol, H}^+}$ 254 (log ϵ 4.36), 340 (4.42), and 397 (3.90) nm; NMR (trifluoroacetic acid): δ 1.13 (t, 3H, *J* = 7 Hz, CH₃CH₂CH₂), 1.90 (m, 2H, CH₃CH₂CH₂), 3.10 (t, 2H, *J* = 7 Hz, H-5), 3.72 (m, 2H, CH₃CH₂CH₂), 4.17 (s, 3H, OCH₃), 4.28 (s, 3H, OCH₃), 4.34 (t, 2H, *J* = 7 Hz, H-6), 6.17 (s, 2H, OCH₂O), 6.97 (s, 1H, H-4), 7.32 (s, 1H, H-13), 7.50 (s, 1H, H-1), and 7.80 (s, 2H, H-11,12); mass spectrum: *m/e* M⁺ 392 (100), 378 (70), 363 (80), 361 (50), 347 (60), 335 (30), 334 (30), 333 (30), 320 (25), 306 (20), 281 (25), and 207 (50).

The VIII-HCl had a melting point of 219–220° (methanol).

Anal.—Calc. for C₂₃H₂₄N₂O₄·HCl: C, 64.37; H, 5.88. Found: C, 63.98; H, 6.07.

Compound IX, C₂₆H₂₂N₂O₄, was obtained as yellow needles, mp 167–169° (methanol-ether); UV: $\lambda_{\text{max}}^{\text{ethanol}}$ 285 (log ϵ 3.95), 328 (3.75), and 370 (3.90) nm; UV: $\lambda_{\text{max}}^{\text{ethanol, H}^+}$ 258 (log ϵ 4.06), 343 (4.06), 395 sh (3.48), and 422 (3.54) nm; mass spectrum: *m/e* M⁺ 426 (100), 411 (40), 395 (30), 335 (30), and 321 (25).

The IX-HCl had a melting point of 186–187° (methanol).

Anal.—Calc. for C₂₆H₂₂N₂O₄·HCl: C, 67.42; H, 5.01. Found: C, 67.17; H, 5.29.

Compound X, C₂₇H₂₄N₂O₄, hydrochloride salt was obtained as gold prisms, mp 160–162° (methanol-water-ether); UV: $\lambda_{\text{max}}^{\text{ethanol, H}^+}$ 236 (log ϵ 4.50), 257 (4.40), 342 (4.38), and 420 (3.90) nm; mass spectrum (free base): *m/e* M⁺ 440 (40), 425 (10), 335 (5), 107 (100), and 106 (98); high-resolution mass spectrum (free base): calc. for C₂₇H₂₄N₂O₄, 440.1731; found *m/e* 440.1749.

Anal.—Calc. for C₂₇H₂₄N₂O₄·HCl·H₂O: C, 65.49; H, 5.50. Found: C, 65.27; H, 5.66.

Conversion of IV to VII—A solution of IV (1 g, 2.85 mmoles) in methyl iodide (3 ml) and acetonitrile (10 ml) was refluxed for 0.5 hr and cooled, and the solvent was evaporated. The residue, after basification with aqueous sodium bicarbonate and chloroform extraction, provided VII in high yield.

8-Berberinylidenemalononitrile (XII)—Malononitrile (0.6 g, 9.1 mmoles) was added to dry tetrahydrofuran (20 ml), followed by sodium hydride (0.2 g, 8.3 mmoles). Following gas evolution, V (1.5 g, 4.08 mmoles) was added, and the mixture was stirred for 30 min. The red mixture was diluted with an equal volume of ether and filtered. The solid was slurried in chloroform and filtered, and the filtrate crystallized upon addition of ether. Recrystallization from methanol afforded 1.1 g (2.75 mmoles, 68%) of XII as reddish-orange crystals, mp 276–277°; IR: $\nu_{\text{max}}^{\text{CHCl}_3}$ 2102 cm⁻¹; UV: $\lambda_{\text{max}}^{\text{ethanol}}$ 255 sh (log ϵ 3.97), 278 (3.85), 345 (3.84), 368 sh (3.73), and 466 (3.51) nm; PMR (trifluoroacetic acid): δ 3.25 (t, 2H, *J* = 7 Hz, H-5), 4.90 (t, 2H, *J* = 7 Hz, H-6), 4.17 (s, 3H, OCH₃), 4.28 (s, 3H, OCH₃), 6.23 (s, 2H, OCH₂O), 6.30 [s, 1H, HC(CN)₂], 7.06 (s, 1H, aromatic H), 7.47 (s, 1H, aromatic H), 7.87 (s, 1H, aromatic H), and 8.06 (s, 2H, H-11,12); mass spectrum: *m/e* M⁺ 399 (100), 384 (20), 359 (60), 334 (40), and 322 (40); high-resolution mass spectrum: calc. for C₂₃H₁₇N₃O₄, 399.1218; found *m/e* 399.1225.

Ethyl 8-Berberinylideneacetoacetate (XIII)—Ethyl acetoacetate (1.2 ml, 8.3 mmoles) was added to a stirred slurry of sodium hydride (0.2 g, 8.3 mmoles) in dry tetrahydrofuran (50 ml). After gas evolution, V (1 g, 2.7 mmoles) was added, and the mixture was stirred for 30 min. The product was partitioned between chloroform and water, the organic layer was dried, and the solvent was evaporated. Trituration with ether gave a product, which was recrystallized from methanol to give 1.04 g (2.15 mmoles, 80%) of XIII as dark-red plates, mp 263–264°; IR: $\nu_{\text{max}}^{\text{KBr}}$ 1620 and 1670 cm⁻¹; UV: $\lambda_{\text{max}}^{\text{ethanol}}$ 232 (log ϵ 4.53), 267 (4.62), 337 (4.33), and 432 (3.73) nm; UV: $\lambda_{\text{max}}^{\text{ethanol, H}^+}$ 232 (log ϵ 4.60), 267 (4.48), 352 (4.38), and 440 (3.72) nm; mass spectrum: *m/e* M⁺ 463 (5), 432 (100), 403 (50), and 358 (80).

Anal.—Calc. for C₂₆H₂₅NO₇·CH₃OH: C, 65.45; H, 5.85. Found: C, 65.37; H, 5.58.

Diethyl 8-Berberinylidenemalonate (XIV)—Salt V (1.5 g, 4 mmoles) was treated with the anion of ethyl malonate as described to yield, after similar workup and treatment with dry methanolic hydrochloric acid, 1.54 g (3.14 mmoles, 79%) of XIV as the hydrochloride salt as light-orange needles, mp >300° (methanol-ether), and red needles of free base, mp 238° (methanol); UV: $\lambda_{\text{max}}^{\text{ethanol}}$ 260 (log ϵ 4.31), 332 (4.15), 402 (3.78), and 428 sh (3.72) nm; mass spectrum: *m/e* M⁺ 493 (2), 448 (2), 421 (10), 390 (30), 376 (40), 361 (45), 346 (40), 334 (100), and 318 (30).

Anal.—Calc. for C₂₇H₂₇NO₈·HCl: C, 61.30; H, 5.11. Found: C, 61.18; H, 4.90.

8-Berberinylacetic Acid (XV) Chloride Salt—A solution of XIV (1.36 g, 2.76 mmoles) in methanol (20 ml) containing concentrated hydrochloric acid (5 ml) was heated at 60° for 1 hr, diluted with water, and extracted with chloroform. The organic layer was dried, filtered, and evaporated. The residue was recrystallized from ethanol-ether to afford 0.85 g (2.16 mmoles, 78%) of XV, mp >300°; IR: $\nu_{\text{max}}^{\text{KBr}}$ 1600, 1720, and 3350 cm⁻¹; UV: $\lambda_{\text{max}}^{\text{ethanol}}$ 272 (log ϵ 4.26), 353 (4.22), 411 sh (3.70), and 430 sh (3.68) nm; PMR (trifluoroacetic acid): δ 3.32 (t, 2H, *J* = 7 Hz, H-5), 4.28 (s, 6H, 2 OCH₃), 4.96 (t, 2H, *J* = 7 Hz, H-6), 5.25 (s, 2H, CH₂COOH), 6.20 (s, 2H, OCH₂O), 7.01 (s, 1H, aromatic H), 7.59 (s, 1H, aromatic H), 8.56 (s, 1H, aromatic H), and 8.14 (s, 2H, H-11,12).

Anal.—Calc. for C₂₂H₂₀ClNO₆·C₂H₅OH: C, 60.58; H, 5.46. Found: C, 60.95; H, 5.38.

8-Canadinylacetic Acid (XVI)—A solution of XV (0.9 g, 2.3 mmoles) in ethanol (50 ml) was treated with excess sodium borohydride, giving an oil that crystallized from methanolic hydrogen chloride solution. Recrystallization from methanol afforded 0.8 g (2.0 mmoles, 87%) of XVI as very light-yellow needles of the hydrochloride salt, mp 212–214°; IR: $\nu_{\text{max}}^{\text{CHCl}_3}$ 1480, 1720, 2400, and 3400 cm⁻¹; UV: $\lambda_{\text{max}}^{\text{ethanol, H}^+}$ 267 sh (log ϵ 3.44) and 287 (3.86) nm; mass spectrum: *m/e* M⁺ 397 (30), 382 (10), 338 (100), 322 (10), 306 (7), 278 (10), 236 (15), 206 (25), 126 (25), and 169 (20).

Anal.—Calc. for C₂₂H₂₃NO₆·HCl: C, 60.89; H, 5.57. Found: C, 61.00; H, 5.88.

8,8-Dimethyldihydroberberine (XVII) Hydroiodide—To a rapidly stirred suspension of magnesium turnings (1 g) in ether (20 ml) was added portionwise a solution of methyl iodide (1 ml) in ether (20 ml) at a rate to maintain vigorous reflux. The mixture was cooled, a suspension of V (1 g, 2.67 mmoles) in ether (20 ml) was added, and the mixture was refluxed for 30 min. The product was poured over ice, mixed with concentrated sulfuric acid (5 ml), and extracted with chloroform. The organic layer was worked up and treated with methanolic hydrogen iodide to furnish 0.78 g (60%) of the hydroiodide salt, mp 222° dec.; PMR (trifluoroacetic acid): δ 2.21 (s, 6H, 2 CH₃), 3.26 (t, 2H, *J* = 7 Hz, H-5), 4.04 (s, 3H, OCH₃), 4.16 (s, 3H, OCH₃), 4.31 (t, 2H, *J* = 7 Hz, H-6), 4.70 (s, 2H, H-13), 6.19 (s, 2H, OCH₂O), 6.93 (s, 1H, H-4), 7.19 (s, 2H, H-11,12), and 7.50 (s, 1H, H-1).

Anal.—Calc. for C₂₂H₂₃NO₄·HI: C, 53.52; H, 4.90. Found: C, 53.12; H, 4.90.

8,8-Diethyldihydroberberine (XVIII) Hydroiodide—By a procedure similar to that described but using ethylmagnesium bromide, XVIII was obtained in 66% yield as orange needles of the hydroiodide salt, mp 290° dec. (methanol).

Anal.—Calc. for C₂₄H₂₇NO₄·HI·CH₃OH: C, 54.25; H, 5.68. Found: C, 54.35; H, 5.39.

8-Benzylberberinium Bromide (XX)—An ethereal V suspension (1 g, 2.7 mmoles) was treated with excess ethereal benzylmagnesium bromide. Workup provided 0.99 g (2.32 mmoles, 86%) of XX as light-red needles, mp 232–233° (methanol); UV: $\lambda_{\text{max}}^{\text{ethanol}}$ 285 (log ϵ 4.04), 365 (3.87), 400 (3.44), and 450 (3.28) nm; PMR (trifluoroacetic acid): δ 3.08 (t, 2H, *J* = 7 Hz, C-5), 3.85 (s, 3H, OCH₃), 4.20 (s, 3H, OCH₃), 4.87 (t, 2H, *J* = 7 Hz, H-6), 5.53 (s, 2H, PhCH₂), 6.15 (s, 2H, OCH₂O), 6.90 (s, 1H, H-4), 7.08–7.43 (m, 5H, ArH), 7.55 (s, 1H, H-1), 8.10 (s, 2H, H-11,12), and 8.55 (s, 1H, H-13); mass spectrum: *m/e* M⁺ 425 (100), 410 (53), 394 (24), and

334 (80); high-resolution mass spectrum: calc. for $C_{27}H_{23}NO_4$, 425.1627; found m/e 425.1615.

8,8-Diethylcanadine (XIX)—A XVIII solution (0.1 g, 0.25 mmole) in ethanol (20 ml) was treated with excess sodium borohydride (0.4 g). Workup and recrystallization from methanol furnished 0.096 g (96%) of XIX as tan plates, mp 168–169°; UV: $\lambda_{max}^{ethanol}$ 233 (log ϵ 4.31) and 288 (3.81) nm; mass spectrum: m/e M^+ 395 (5), 367 (100), 335 (10), 220 (35), and 175 (40); high-resolution mass spectrum: calc. for $C_{24}H_{29}NO_4$, 395.2096; found m/e 395.2125.

8-Benzylcanadine (XXI)—Sodium borohydride reduction of XX (1 g, 2.3 mmoles) in ethanol yielded 0.98 g (99%) of the known XXI, mp 165–166° (methanol) [lit. (5) mp 163–165°].

REFERENCES

(1) M. Shamma, "The Isoquinoline Alkaloids," Academic, New York, N.Y., 1972, pp. 268–314.

(2) M. Shamma and J. L. Moniot, "Isoquinoline Alkaloids Research, 1972–1977," Plenum, New York, N.Y., 1978, pp. 209–259.

(3) R. D. Haworth, W. H. Perkin, Jr., and H. S. Pink, *J. Chem. Soc.*, 1925, 1709.

(4) A. C. Santoso and P. Adkilen, *J. Am. Chem. Soc.*, **54**, 2923 (1932).

(5) J. R. Gear and I. D. Spenser, *Can. J. Chem.*, **41**, 783 (1963).

ACKNOWLEDGMENTS

Supported by Grant HL-12971 from the National Heart, Lung and Blood Institute, U.S. Public Health Service, National Institutes of Health.

A. H. Abd el Rahman, of the Department of Chemistry, Mansura University, Mansura, Egypt, was the recipient of a postdoctoral fellowship from the Egyptian government.

Comparative Studies on Eight Dissolution Methods Using 21 Commercial Chloramphenicol Tablets and a Nondisintegrating Benzoic Acid Tablet

HIROYASU OGATA*, TOSHIO SHIBAZAKI, TETSUO INOUE, and AKIRA EJIMA

Received April 25, 1978, from the Division of Drug, National Institute of Hygienic Sciences, 1-18-1, Kamiyoga Setagaya-ku, Tokyo, Japan. Accepted for publication November 17, 1978.

Abstract □ Eight dissolution methods (beaker, rotating basket, oscillating basket, solubility simulator, rotating flask, and column) were evaluated using 21 commercial film-coated chloramphenicol (I) tablets and a nondisintegrating benzoic acid (II) tablet. The relative agitating intensities obtained from different dissolution methods were compared through the relative zero-order nondisintegrating tablet dissolution rate constants. Correlation coefficients between I dissolution rate parameters (lag time, T_{20} , T_{50} , and T_{80}) were determined. Significant correlation was observed for the lag time among seven methods, and all pairwise regression lines passed through zero except one. The regression line slopes reflected the relative destructive force intensities produced by each dissolution method on the coated I tablet films. The seven dissolution methods could be classified into two main groups according to correlations of four dissolution rate parameters. This classification criterion agreed well with that based on the agitation method. However, dissolution methods may not be interchangeable even though they belong to the same dissolution method group.

Keyphrases □ Dissolution testing systems—eight systems compared, chloramphenicol tablets and powder, benzoic acid tablet □ Dissolution rates—chloramphenicol tablets and powder, benzoic acid tablet □ Chloramphenicol—dissolution rates, tablets and powder, eight testing systems compared □ Benzoic acid—dissolution rate, tablet, eight testing systems compared

Since the dissolution rate was first recognized as a significant factor in determining *in vivo* drug bioavailability, many methods for testing solid dosage form dissolution have been reported (1, 2). These methods differ in hydrodynamic properties, agitating intensities, and mechanical destructive forces to the intact drug. There are three basic dissolution testing devices (2, 3): the stirred-tank reactor with a mechanical agitator such as Levy's beaker method (4) or the rotating-basket method (5), the rotating vessel reactor such as the rotating flask (6) or the

solubility simulator¹, and the stream reactor such as the column method (7–9).

Several investigators compared the relative agitating intensities or hydrodynamic properties of selected dissolution devices by using a nondisintegrating model tablet (10–12). Bathe *et al.* (3) studied dissolution rates by beaker, rotating-basket, and flow column methods under eight different conditions. However, detailed comparisons of disintegrating tablet dissolution methods have not been made.

In the present study, eight representative dissolution testing methods were compared and evaluated. They can be divided into three categories: (a) stirrer-tank reactor type (beaker, rotating basket, and oscillating basket), (b) rotating-vessel reactor type (rotating flask and solubility simulator¹), and (c) stream reactor type (column). Disintegrating tablets—21 chloramphenicol (I) brands available in Japan—and nondisintegrating tablets—benzoic acid (II)—were used as markers. The two drugs were selected because of their moderate solubilities in acidic solutions (2–4 mg/ml).

EXPERIMENTAL

Materials—Twenty-one different sugar-coated I tablets were obtained from 16 manufacturers in Japan. Tablets A–E each contained 50 mg of I while Tablets F–U each contained 250 mg of I. A powder of I² was included for comparison. Nondisintegrating and uncoated tablets containing 100 mg of II³ were used.

¹ Sartorius-Membranfilter GmbH, Göttingen, West Germany.

² Yamanouchi Pharmaceutical Co., Tokyo, Japan.

³ Supplied by Dr. Ikeda and Dr. Nishimura, Sankyo Co., Tokyo, Japan.