Antibacterial and Antifungal Activity of Liriodenine and Related Oxoaporphine Alkaloids

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Abstract □ Liriodenine was evaluated for its antibacterial and antifungal activity against several microorganisms. Other related oxoaporphine alkaloids also were evaluated. Attempts to prepare oxoaporphine alkaloids from *N*-acetylnoraporphines were unsuccessful, but an unexpected phenanthrene alkaloid was obtained. A novel *N*-demethylation reaction was noted when oxoglaucine methiodide and liriodenine methiodide were treated with alumina.

Keyphrases □ Liriodenine—antibacterial and antifungal activity □ Oxoaporphine alkaloids—preparation and evaluation for antimicrobial activity □ Antimicrobial activity—evaluation of liriodenine and related oxoaporphine alkaloids

In spite of the development of many clinically effective antibiotics for the treatment of bacterial diseases, there are relatively few clinically effective antifungal antibiotics, particularly for systemic fungal infections. Amphotericin B and flucytosine are the only effective antifungal agents against systemic mycoses. Drug therapy for superficial as well as systemic fungal infections was reviewed previously (1, 2).

As part of a program of random screening of higher plants for antibacterial and antifungal activity, liriodenine (I), an oxoaporphine alkaloid isolated from the heartwood of *Liriodendron tulipifera*, was identified as potentially useful as an antifungal antibiotic (3). This report describes some further biological data for liriodenine and the preparation and evaluation of several other oxoaporphine alkaloids.

RESULTS AND DISCUSSION

Liriodenine (I) has been isolated from the heartwood of *L. tulipifera* (3), but a synthetic method was desirable for the preparation of larger quantities. Liriodenine was synthesized as described previously (4), and references to the preparation of intermediates and modifications are noted under *Experimental*. Compound I previously was shown to have activity against Gram-positive bacteria, acid-fast bacteria, and several fungal organisms (3). Further evaluation of I seemed warranted; with larger quantities available, it became possible to explore a wide range of biological activities.

Compound I was tested against four additional fungi (Table I) and showed good activity against *Trichophyton mentagrophytes* and *Syncephalestrum racemosum* compared to griseofulvin and candicidin. Its antimicrobial spectrum of activity also was evaluated (Table II) and agreed well with the results reported previously (3) and in Table I. The activity of I against a number of plant diseases was evaluated; it showed good activity against barley net blotch, chocolate spot of broad beans, and rice blast (Table III). Compound I also was relatively nontoxic in acute toxicity tests[1].

Oxoglaucine (II), another oxoaporphine alkaloid from *L. tulipifera*, is devoid of antifungal and antibacterial activity, while its methiodide salt does show antimicrobial activity, particularly against *Candida albicans* (3). Liriodenine methiodide also is more active against *C. albicans* than I (3). These results prompted a study of the antimicrobial activities of other oxoaporphine alkaloids.

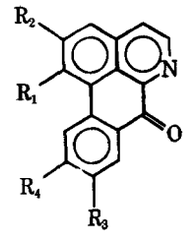
Corunnine (III) was prepared by refluxing the purified methiodide salt of II in acetone as described previously (5). A minor product from this reaction was II, which was thought to be generated during purification of the reaction product (filtration through basic alumina). Since II was not present in the starting material (TLC), this finding suggested that the alumina was effecting *N*-demethylation of some of the unreacted methiodide salt. Oxoglaucine methiodide was stirred overnight in a slurry of basic alumina with methanol–chloroform as the solvent, and II was produced in a 75% yield along with III.

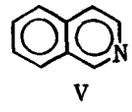
Liriodenine methiodide also was exposed to basic alumina in a similar manner, and I was obtained in a 85% yield along with a small amount of a blue alkaloid, which was identified as 2-*N*,*O*-dimethylliriodendronine[2] (IV) by its UV and PMR spectral characteristics (6, 7). The identity was

Table I—Minimum Inhibitory Concentration of Liriodenine for Selected Fungi [a]

Compound	*Trichophyton mentagrophytes* (ATCC 9972)	*Aspergillus niger* (ATCC 16888)	*Syncephalestrum racemosum* (ATCC 18192)	*Mucor griseocyanus* (ATCC 1207)
Liriodenine (I)	2 (2)	25 (12)	3.1 (3.1)	25 (100)
Candicidin	50 (25)	3 (2)	0.4 (0.8)	0.2 (0.8)
Griseofulvin	2 (1)	100 (100)	25 (50)	100 (100)

[a] The values expressed are in micrograms per milliliter (figures in parentheses are duplicate runs). The fungi were grown in mycophil (BBL) broth and incubated at 30°, with readings taken at 48 hr as described previously (3).





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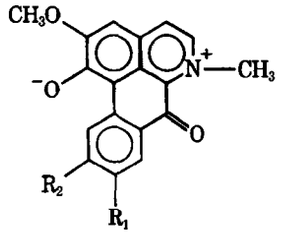
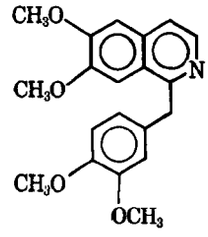
I: $R_1 + R_2 = OCH_2O$, $R_3 = R_4 = H$
II: $R_1 = R_2 = R_3 = R_4 = OCH_3$
XI: $R_1 = R_2 = OCH_3$, $R_3 = R_4 = H$
XII: $R_1 = R_2 = OCH_3$, $R_3 + R_4 = OCH_2O$
XIII: $R_1 + R_2 = R_3 + R_4 = OCH_2O$



III: $R_1 = R_2 = OCH_3$
IV: $R_1 = R_2 = H$



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[1] The LD_{50} values in mice were >400 mg/kg ip and 120 mg/kg iv.

[2] The isolation of IV and III by alumina treatment of the methiodide salts of I and II, respectively, suggests that alumina may be involved in their production. The authors of Ref. 5 proposed a mechanism to account for the production of III; but since alumina was used in the purification steps, some questions now arise about this proposed mechanism. A complete explanation awaits further study.

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Table II—Antimicrobial Spectrum of Activity of Liriodenine [a]

Organism (ATCC Number)	Minimum Inhibitory Concentration, μg/ml
Aerobacter cloacae (13047)	>64
Alcaligenes faecalis (8750)	>64
Bacillus subtilis (6051)	1
Escherichia coli (11775)	>64
Herellea vaginicola (19683)	8
Klebsiella sp. (4352)	8
Pseudomonas aeruginosa (15442)	>64
Pseudomonas aeruginosa (9721)	>64
Pseudomonas aeruginosa (10145)	>64
Salmonella typhimerium (13311)	>64
Staphylococcus aureus (6538)	2
Staphylococcus aureus (12600)	4
Staphylococcus epidermidis (155)	2
Staphylococcus epidermidis (14990)	2
Candida albicans (10231)	8
Aspergillus niger (16404)	>64
Trichophyton mentagrophytes (8757)	1
Trichophyton mentagrophytes (9129)	2
Trichophyton rubrum (10218)	2
Trichophyton rubrum (14001)	1

[a] Data were obtained using standard agar incorporation (serial dilution) by Westwood Pharmaceutical Inc. The bacteria were grown on Mueller-Hinton agar (BBL) and incubated at 32°, with readings taken at 48 hr; the fungi were grown on YM agar (BBL) and incubated at 28°, with readings taken at 48 hr.

Table III—Antimicrobial Activity of Liriodenine against Plant Pathogenic Microorganisms [a]

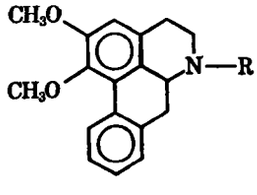
Organism (Common Name)	Liriodenine	Mancozeb	Benomyl
1 *Helminthosporin teres* (barley net blotch)	A (B)	A (B)	E (E)
2 *Botrytis fabae* (chocolate spot of broad beans)	A (B)	E (E)	A (A)
3 *Erysiphe polygoni* (bean powdery mildew)	E (E)	E (E)	A (A)
4 *Plasmopora viticola* (grape downy mildew)	A (B)	A (B)	E (E)
5 *Piricularia oryzae* (rice blast)	A (B)	A (A)	A (A)
6 *Phytophthora infestans* (tomato late blight)	E (E)	A (A)	E (E)
7 *Puccina graminia-tritici* (wheat stem rust)	E (E)	A (A)	E (E)

[a] The testing was performed at Rohm and Haas Co., and results are expressed as percent disease control: A, 97–100%, B, 90–96%; C, 70–89%; D, 50–69%; and E, <50%. The compounds were applied at 300 ppm as a foliar spray. The data appearing in parentheses are at 38 ppm. The treated plant foliage was allowed to dry and then was inoculated with plant pathogenic organisms within 30 hr of the initial treatment. Plants were maintained under environmental conditions that favor optimal disease development, and readings were made at appropriate time intervals after treatment depending upon the disease: 1, 6–7 days; 2, 66–68 hr; 3, 8–10 days; 4, 7 days; 5, 7–8 days; 6, 5–6 days; and 7, 14 days. Mancozeb (Rohm and Haas) and benomyl (Dupont) are commercial standards included for comparison. Liriodenine is slightly less active than the commercial standards at lower rates of application (figures in parentheses).

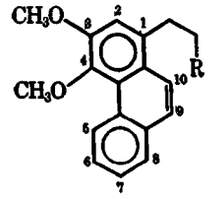
established by direct comparison with an authentic sample. It is apparent from these two examples that alumina treatment of the methiodide salts of I and II causes *N*-demethylation. The methiodide salts of isoquinoline (V) and papaverine (VI) were treated with alumina, but no demethylation was observed[3]. No activity was observed for III and IV when tested in the antimicrobial assay (3).

To evaluate the antimicrobial spectrum of other oxoaporphine alkaloids, it was felt that a number of these compounds could be obtained by chemical conversions of *N*-acetylnoraporphines available from previous work (8, 9). *N*-Acetylnornuciferine (VII) (9) was selected as a model since it was available in the largest quantities. Hydrolysis of VII and oxidation were expected to yield the known oxoaporphine lysicamine (XI). Base-catalyzed hydrolysis was believed to be more desirable than acid-catalyzed hydrolysis since the methoxyl groups should be unaffected.

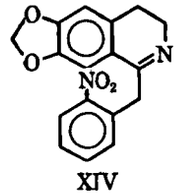
Attempts to hydrolyze VII using potassium *tert*-butoxide (10), lithium alkylamine (11), or Grignard reagents (12) were unsuccessful, so acidic hydrolytic procedures were considered. Selective hydrolysis of the *N*-acetyl function in colchicine using methanol–hydrochloric acid was reported previously (13). Refluxing VII in methanol–hydrochloric acid for 24 hr resulted in a high yield of a single crystalline product. Its melting point was considerably different from that reported for the expected VIII·HCl (14), and examination of the spectral properties clearly showed that



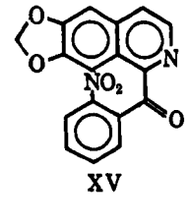
VII: R = $COCH_3$
VIII: R = H



IX: R = NH_2
X: R = $N(CH_3)_2$



XIV



XV

[3] A full explanation of the demethylation of oxoaporphines should follow further studies.

the product was not VIII. The spectral data were more consistent with identification of the product as bisnoratherosperminine (IX) hydrochloride, a previously unreported alkaloid.

The molecular formula for IX·HCl was confirmed by combustion analysis and high-resolution mass spectrometry. The IR spectrum showed no carbonyl band, and the UV spectrum was characteristic of phenanthrene alkaloids and not aporphines (15, 16). The PMR spectrum showed a highly deshielded proton at δ 9.37, characteristic of H-5 in phenanthrene alkaloids (16). The ^{13}C-NMR spectral data were in agreement for IX. Compound IX was converted to atherosperminine (X) and was characterized as the picrate salt, having physical and spectral properties similar to those reported for atherosperminine (X) picrate (17). A direct comparison showed the two samples to be indistinguishable. Compound IX apparently arises from a Hofmann-like elimination reaction, although a complete explanation of the mechanism of this reaction remains to be established. This paper is the first report of the conversion of an *N*-acetylnoraporphine alkaloid to a phenanthrene alkaloid.

Since suitable conditions could not be worked out for the conversion of the *N*-acetylnoraporphines to the corresponding oxoaporphines, the complete biological evaluation of a number of oxoaporphines was not possible. However, authentic samples of lysicamine (XI) and oxonantenine (XII) were obtained, and cassameridine (XIII) was synthesized as described in the literature (18). Compounds XI–XIII were tested in the qualitative and quantitative antimicrobial screen (3), and the quantitative results are shown in Table IV.

EXPERIMENTAL[4]

Synthesis of Liriodenine (I)—The method used was essentially the same as that reported previously (4). *o*-Nitrophenylacetic acid was obtained commercially[5] or was conveniently synthesized (19). β-(3,4-Methylenedioxyphenyl)ethylamine was synthesized by catalytic reduction of 3,4-methylenedioxy-β-nitrostyrene (20), which had been prepared from piperonal and nitromethane (21). β-(3,4-Methylenedioxyphenyl)ethyl-*o*-nitrophenylacetamide was synthesized from β-(3,4-methylenedioxyphenyl)ethylamine and *o*-nitrophenylacetic acid (22) and then cyclized to the dihydroisoquinoline (XIV) using phosphorus

[4] Melting points were determined on a Fisher-Johns 355 digital melting-point analyzer and are uncorrected. Elemental analyses were performed by Scandinavian Laboratories, Herlev, Denmark. IR spectra were determined on a Beckman IR-33 or Perkin-Elmer model 257 recording spectrophotometer. UV spectra were determined on a Beckman Acta III spectrophotometer. PMR spectra were recorded using a JEOL C-60HL (60-MHz) spectrometer, while ^{13}C-NMR spectra were obtained on a JEOL JNM-FX60 (15.03-MHz) spectrometer with tetramethylsilane as the internal standard. TLC was performed on either precoated silica gel (0.25 mm) or alumina (0.25 mm) plates, using Dragendorff's reagent or UV light for visualization.

[5] Aldrich Chemical Co.

Table IV—Minimum Inhibitory Concentration of Lysicamine, Oxonantenine, and Cassameridine for Selected Microorganisms [a]

Compound	*Staphylococcus aureus* (ATCC 6538)	*Mycobacterium smegmatis* (ATCC 607)	*Trichophyton mentagrophytes* (ATCC 9972)	*Saccharomyces cerevisiae* (ATCC 9763)
Lysicamine (XI)	25	12.5	12.5	12.5
Oxonantenine (XII)	12.5	6.3	12.5	12.5
Cassameridine (XIII)	50	25	25	25
Amphotericin B	—	—	12.5	0.8
Streptomycin sulfate	6.3	1.6	—	—

[a] The values expressed are in micrograms per milliliter. The bacteria were grown in eugon broth (BBL) and incubated at 37°, and readings were taken at 24 hr. The fungi were grown in mycophil broth (BBL) and incubated at 30°, and readings were taken at 48 hr as described previously (3).

oxychloride in acetonitrile (22, 23). The dihydroisoquinoline then was oxidized directly to XV rather than by two-step oxidation (4).

A mixture of 100 mg of XIV, 250 mg of potassium dichromate, and 3 ml of 70% acetic acid was refluxed for 4 hr. The cooled reaction mixture was diluted with 2 ml of water, neutralized with 10 ml of saturated sodium bicarbonate solution, and extracted with chloroform (3 × 20 ml). The residue from the chloroform extract was purified by chromatography over basic alumina [benzene–chloroform (1:1)] to give 61 mg of XV, mp 249–251° dec. [lit. (4) mp 252° dec.].

The synthesis was completed by Pschorr cyclization of XV to I as described previously (4). The synthesis of I using the outlined procedure was accomplished in a 10% overall yield [lit. (4) 4% yield] from piperonal and *o*-nitrophenylacetic acid. All of the intermediates were tested, but none showed any antimicrobial activity.

Corunnine (III)—*Method A*—A 100-mg sample of oxoglaucine (II) methiodide[6] (3) was refluxed in 10 ml of acetone for 3 days. Evaporation of the solvent and chromatography over basic alumina (grade III, 10 g) using 4% methanol in chloroform gave a green eluate, which, upon concentration, gave 76 mg of corunnine (III), mp 254–255° [lit. (5) mp 255–257°]. The UV data in methanol and methanol–hydrochloric acid as well as the PMR data were consistent with the data reported for III (5). A small amount of a yellow band was collected from the column, and it appeared to be II by TLC.

Method B—A solution of 100 mg of II, 3.5 ml of iodomethane, and 5 ml of dry benzene was refluxed for 6 hr. The solution was cooled and evaporated, and the residue was examined by TLC (6% methanol in chloroform on silica gel G). It showed no evidence of II. It then was chromatographed as described. The yellow band was collected and, upon evaporation, gave 14 mg of II, which was identified by direct comparison with an authentic sample (melting-point, mixed-melting point, and TLC analyses and superimposable IR spectra). The green band gave 10 mg of III, mp 254–255°.

Method C—Oxoglaucine methiodide (100 mg) was dissolved in 4% methanol–chloroform. To this solution was added 4 g of basic alumina (grade III), and the slurry was stirred for 22 hr at room temperature. Then the slurry was filtered, and the alumina was washed thoroughly with additional solvent. The combined solutions were evaporated, and the residue was chromatographed over silica gel 60 with 2% methanol in chloroform as the eluent. The orange-yellow band was collected and identified as oxoglaucine (53 mg). The green band was eluted with 4% methanol in chloroform and gave 23 mg of III.

Treatment of Liriodenine Methiodide with Aluminum Oxide—Liriodenine methiodide (800 mg) was dissolved in 4% methanol in chloroform (no I was shown to be present by TLC), 30 g of basic alumina (grade III) was added, and the slurry was stirred overnight (18 hr). Then the slurry was washed thoroughly with methanol–chloroform. The combined washings were evaporated, and the residue was chromatographed over silica gel G 60 (230–400 mesh) with 2% methanol in chloroform as the eluent. The yellow band was collected and, upon concentration, gave 464 mg of I, which was identified by direct comparison with an authentic sample (melting-point determination and superimposable IR spectra).

The eluent was changed to 4% methanol in chloroform, and the blue band, collected upon evaporation and crystallization from methanol, gave 20 mg of IV as blue needles, mp 284–286° dec. [lit. (7) mp 275–278°]. The identity of IV was established by direct comparison with an authentic sample (melting-point, mixed melting-point, and IR, UV, and TLC comparisons).

Treatment of Isoquinoline Methiodide and Papaverine Methiodide with Aluminum Oxide—Isoquinoline (V) methiodide, mp 158–159° [lit. (26) mp 159°], and papaverine (VI) methiodide, mp 193–195° [lit. (27) mp 195°], were each treated with basic alumina as described for oxoglaucine methiodide and liriodenine methiodide. The reaction solutions did not show any free bases by TLC (silica gel G and 6% methanol in chloroform).

Hydrolysis of *N*-Acetylnornuciferine (VII) with Methanol-Hydrochloric Acid—A 50-mg sample of VII was dissolved in 8 ml of methanol. Concentrated hydrochloric acid (6 ml) was added, and the solution was refluxed for 24 hr. The cooled solution was evaporated to remove the methanol, after which 45 mg of IX·HCl was obtained, mp 217–220°. Crystallization from methanol+ether gave 31 mg of IX·HCl, mp 221–222°; IR (KBr): ν_{max} 3500 (broad) and 1600 cm^{-1}; UV (methanol): λ_{max} (log ϵ) 311 (4.06), 304 (4.06), 274 (4.06), 256 (4.78), 249 (4.65), 233 (4.32), and 212 (4.24) nm; PMR (D_2O): δ 9.37 (m, 1H, H-5), 7.61 (m, 5H, aromatic H), 7.17 (s, 1H, H-2), 3.98 (s, 3H, C-3 OCH_3), 3.78 (s, 3H, C-4 OCH_3), and 3.27 (m, 4H, CH_2CH_2); ^{13}C-NMR (methanol-d_4): δ 32.3 (t, $CH_2CH_2NH_2$), 41.6 (t, $CH_2CH_2NH_2$), 57.2 (q, C-3 OCH_3), 60.2 (q, C-4 OCH_3), 116.8 (d, C-2), 122.7 (s, C-4a), 147.8 and 152.2 (s, C-3 and C-4) and signals at 122.7 (d), 126.4 (d), 127.3 (d), 127.5 (d), 127.6 (d), 128.8 (d), 129.2 (d), 130.7 (s), 131.0 (s), and 134.1 (s) representing the remaining aromatic carbon atoms; mass spectrum: *m/e* 281.137 [M^+] ($C_{18}H_{19}NO_2$ requires 281.142). Compound IX did not show any antimicrobial activity.

Anal.—Calc. for $C_{18}H_{19}NO_2$·HCl: C, 68.02; H, 6.34; N, 4.41. Found: C, 67.64; H, 6.23; N, 4.04.

Atherosperminine (X)—Bisnoratherosperminine (IX) hydrochloride (15 mg) was dissolved in 1 ml of methanol and 0.02 ml of 37% formaldehyde. To this solution was added 6 mg of sodium borohydride. Additional portions of sodium borohydride (6 mg) and 37% formaldehyde (0.02 ml) were added after 4 and 7 hr. After 24 hr, the solution was acidified with acetic acid and evaporated to dryness. The solid residue was dissolved in chloroform (5 ml), washed once with water (5 ml), dried, and evaporated to dryness (17 mg).

This residue in 0.5 ml of methanol was treated with a methanolic solution of picric acid (11 mg/2 ml) and, upon standing, yielded 19 mg of the picrate salt of X, mp 180–181° [lit. (17) 186–188°]. An authentic sample of atherosperminine picrate had a melting point of 179–181° and was not depressed upon admixture with the prepared sample. The picrates had identical IR spectra. The PMR spectra and TLC properties [silica gel with chloroform–methanol–acetic acid (75:20:5)] of the bases (liberated from the picrates by filtration through alumina) were the same.

Synthesis of Cassameridine (XIII)—The synthesis was accomplished as described in the literature (18), although the yields of several reactions were quite low using this procedure. The final product (XIII) had a melting point of 310–313° [lit. (18) mp 301–302°] and a molecular ion at *m/e* 319. It also had IR and UV data consistent with those reported previously (18).

REFERENCES

(1) G. S. Kobayashi and G. Medoff, *Ann. Rev. Microbiol.*, **31**, 291 (1977).

(2) R. P. Knott and B. Rowles, *PharmIndex*, **20**, 6 (1978).

(3) C. D. Hufford, M. J. Funderburk, J. M. Morgan, and L. W. Robertson, *J. Pharm. Sci.*, **64**, 789 (1975).

(4) W. I. Taylor, *Tetrahedron*, **14**, 42 (1961).

(5) I. Ribas, J. Sueiras, and L. Castedo, *Tetrahedron Lett.*, **1971**, 3093.

(6) P. D. Senter and C.-L. Chen, *Phytochemistry*, **16**, 2015 (1977).

(7) J. M. Sad, M. J. Mitchell, and M. P. Cava, *Tetrahedron Lett.*, **1976**, 601.

(8) C. D. Hufford and M. J. Funderburk, *J. Pharm. Sci.*, **63**, 1338 (1974).

(9) C. D. Hufford, *Phytochemistry*, **15**, 1169 (1976).

(10) P. G. Gassman, P. K. G. Hodgson, and R. J. Balchunis, *J. Am. Chem. Soc.*, **98**, 1275 (1976).

[6] Oxoglaucine (II) was obtained in a high yield by lead tetraacetate oxidation of glaucine (24). Glaucine was available from the heartwood of *L. tulipifera* (3) or by methylation (25) of boldine (Sigma Chemical Co.).

(11) A. Patchornik, M. Wilchek, and S. Sarid, *ibid.*, **86**, 1457 (1964).
(12) H. A. Staab and E. Jost, *Chem. Ber.*, **655**, 90 (1962).
(13) H. Fernholz, *Angew. Chem.*, **65**, 319 (1953).
(14) R. F. Borch, *Tetrahedron Lett.*, **1968**, 61.
(15) H. Guinaudeau, M. Leboeuf, and M. Cave, *Lloydia*, **38**, 275 (1975).
(16) M. Shamma, "The Isoquinoline Alkaloids," Academic, New York, N.Y., 1972, chap. 14, pp. 262, 263.
(17) I. R. C. Bick and G. K. Douglas, *Aust. J. Chem.*, **18**, 1997 (1965).
(18) F. N. Lahey and K. F. Mak, *Tetrahedron Lett.*, **1970**, 4311.
(19) L. Marion and V. Gassie, *J. Am. Chem. Soc.*, **66**, 1990 (1944).
(20) D. D. Wagner, A. I. Rachlin, and S. Teitel, *Synth. Commun.*, **1**, 47 (1971).
(21) T. R. Govindachari, S. Rajadurai, C. V. Ramadas, and N. Vishwanathan, *Ber.*, **93**, 360 (1959).
(22) G. Barger and G. Weitnauer, *Helv. Chim. Acta*, **22**, 1036 (1939).
(23) C. D. Hufford and J. M. Morgan, *J. Org. Chem.*, **41**, 375 (1976).
(24) L. Castedo, R. Suau, and A. Mourino, *Heterocycles*, **3**, 449 (1975).
(25) V. Preininger, R. S. Thakur, and F. Santavy, *J. Pharm. Sci.*, **65**, 294 (1976).
(26) M. Harris and C. Pope, *J. Chem. Soc.*, **121**, 1030 (1922).
(27) B. Shizhov, *Sov. Pharm. Res.*, **3**, 90 (1958).

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Formylation of Glucose by Cefamandole Nafate at Alkaline pH

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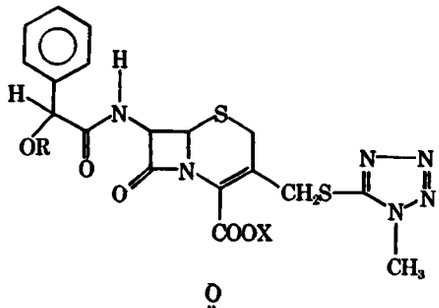
Abstract □ An increase in the hydrolysis rate of the formyl moiety of cefamandole nafate was observed when the commercial product was reconstituted in 5% dextrose in water relative to the rate when the formulation was dissolved in water for injection or in 0.9% saline. The increase in ester cleavage was the result of nucleophilic attack of glucose on the formyl ester moiety. This transesterification produced small amounts of D-glucose-6-formate and other D-glucose diformates. The formation of these products is of no clinical significance since the antibiotic potency and stability of cefamandole are unaffected and no toxicological differences were observed in animal studies or in clinical trials when formulated cefamandole nafate was administered with or without glucose.

Keyphrases □ Cefamandole nafate—hydrolysis in presence of glucose □ Glucose—effect on hydrolysis of cefamandole nafate □ Hydrolysis—cefamandole nafate, effect of glucose

Cefamandole nafate (I*a*) is the formyl ester of the sodium salt of cefamandole [7-D-mandelamido-3-[[(1-methyl - 1*H* - tetrazol - 5 - yl)thio]methyl] - 3 - cephem - 4 - carboxylic acid, I*b*]. Cefamandole nafate is converted rapidly *in vivo* (1) and *in vitro* (2) to cefamandole. Against Gram-positive organisms, cefamandole has microbiological activity comparable to that of cephalothin and cefazolin; against Gram-negative bacteria, cefamandole clearly is superior to these antibiotics both in its activity and its spectrum (3).



I*a*: R = CH, X = Na
I*b*: R = H, X = H

BACKGROUND

Cefamandole nafate, which may be administered either intramuscularly or intravenously, maintains microbiological potency in commercial intravenous fluids for >96 hr at 4°. However, an increase in the rate and extent of hydrolysis of the formyl moiety has been observed (Fig. 1) when formulated cefamandole nafate[1] is reconstituted in commercial 5% dextrose in water relative to the rate when the formulation is dissolved in water for injection or in 0.9% saline. This change in the hydrolysis rate of the formyl moiety is related to the glucose concentration (Fig. 2) and occurs only in the formulated product.

A similar two- to threefold increase in the hydrolysis rate of phenyl acetates in the presence of glucose was reported previously (4). This increase was presumed to be the result of nucleophilic attack on the carbonyl carbon by the hemiacetal alkoxide ion derived from glucose; however, neither glucose-1-acetate nor any other glucose ester was isolated.

Glucose and other carbohydrates have been implicated in the biological inactivation of penicillins at pH 7.4 and higher (5, 6). This inactivation was shown to be the result of nucleophilic attack on the β-lactam ring, resulting in the formation of penicilloyl sugar esters, which subsequently hydrolyze to penicilloic acids (7, 8).

The purposes of this study were to define the role of glucose in the

[1] Mandol, Eli Lilly and Co., Indianapolis, Ind. Vials equal to 1 g of cefamandole (free acid) contain 1.11 g of cefamandole nafate and 0.063 g (0.28 mole equivalent) of sodium carbonate.

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