Cactus Alkaloids XXI: β-Phenethylamines from *Dolichothele sphaerica*

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Abstract □ Studies concerning the isolation and biosynthesis of an imidazole alkaloid, dolichotheline (*N*-isovalerylhistamine), in the cactus *Dolichothele sphaerica* (Dietr.) Br. and R. were previously reported. In the current investigation, chromatographic analysis (TLC) detected additional alkaloids; however, these compounds were β-phenethylamines rather than imidazoles. Preparative TLC aided in the crystallization of five of these compounds: *N*-methylphenethylamine hydrochloride, *N*-methyltyramine hydrochloride, synephrine hydrochloride, β-*O*-methylsynephrine hydrochloride, and β-*O*-ethylsynephrine hydrochloride. The last compound was shown to be an extraction artifact of synephrine. This is the first report of the isolation of *N*-methylphenethylamine from the Cactaceae, but the other natural compounds were all previously isolated from other cactus species.

Keyphrases □ Cactus alkaloids—isolation, identification of five β-phenethylamines from *Dolichothele sphaerica* □ *Dolichothele sphaerica* (Dietr.) Br. and R.—isolation, identification of five β-phenethylamines □ β-Phenethylamine alkaloids—isolation, identification from *Dolichothele sphaerica*

The known cactus alkaloids are generally either β-phenethylamines or the related tetrahydroisoquinolines. An exception is the imidazole alkaloid dolichotheline, 4(5)-[2-*N*-isovalerylaminoethyl]imidazole or *N*-isovalerylhistamine. This compound was isolated (1, 2) from *Dolichothele sphaerica* (Dietr.) Br. and R., a small cactus indigenous to southern Texas and northern Mexico. The biosynthesis of this unusual alkaloid was demonstrated (3, 4) to involve amide formation between histamine and isovaleric acid, with the latter compound likely arising naturally from leucine catabolism. Recently, this plant was also found (5) to produce aberrant imidazole alkaloids, such as *N*-isocaproylhistamine and 4(5)-[*N*-isovalerylaminomethyl]imidazole, when administered the proper exogenous precursors.

Earlier reports referred to the presence of "toxic" alkaloids in this species (6) and to ethnobotanical relationships of *Dolichothele* species with *Lophophora* through the common name of "peyote" (7–9). The present phytochemical studies with *D. sphaerica* were undertaken to isolate and identify additional unusual alkaloids that might have medicinal or psychotropic potential.

EXPERIMENTAL

Plant Material—Specimens of the cactus were purchased[1], and identification was made by comparison with a literature description[2] (10). Since the genus *Dolichothele* is not universally recognized, the botanical synonym, *Mammillaria longimamma* DC. var. *sphaerica* K. Brandegee as described by Benson (11), was included in the identification. The cacti were sliced, frozen, freeze dried (87% moisture) at temperatures not exceeding 30°, and ground to a powder using a 2-mm. sieve on a Fitzpatrick mill.

Preparation of Crude Alkaloid Fractions—The powdered plant material (617 g.) was defatted, basified, and extracted *via* chloroform percolation (12, 13). Condensing the filtered chloroform extract on a flash evaporator[3] and then under rotary vacuum evaporation produced a thick syrup, which was extracted with six successive 200-ml. portions of 1 *N* hydrochloric acid. The aqueous extracts were filtered, combined, and extracted six times with 1-l. portions of both chloroform and ether. These organic extracts were combined, dried over anhydrous sodium sulfate, and condensed under rotary vacuum to a residue containing primarily nonalkaloidal material (Fraction B).

Following basification of the aqueous solution to pH 8.5 with sodium hydroxide, the solution was extracted with 3 l. each of chloroform and ether (in 1-l. portions). Repeated extractions at pH 9.5 and 10.5 resulted in combined extracts totaling 18 l., which were condensed with the flash and rotary vacuum evaporators to a volume of 5 ml. containing the bulk of the alkaloids (Fraction A).

Traces of organic solvents were removed from the aqueous solution by placing it under rotary vacuum evaporation at 40° for 30 min.; then it was freeze dried. The remaining salt residue was extracted with portions of 10% ethanol in chloroform until the eluates were colorless. The combined eluates were filtered and condensed under rotary vacuum evaporation to yield the water-soluble alkaloids (Fraction C).

Analysis of crude alkaloid Fractions A, B, and C with analytical TLC detected dolichotheline and four unknown alkaloids in Fractions A and C; Fraction B contained traces of dolichotheline mixed with nonalkaloidal material. Fractions A and C were combined, and the resulting ethanolic solution was separated into phenolic and nonphenolic portions using a basic ion-exchange column[4] as previously described (12).

TLC—Previously utilized TLC methods were used analytically to provide a tentative identification of alkaloids in the extracts and preparatively to separate the components (12–15). Analytical TLC plates were purchased[5], and preparative plates were prepared with a 1-mm. layer of silica gel PF_{254}[6].

Analytical separations were achieved by the use of the following solvent systems: Solvent A, ethyl acetate–methanol–28% ammonium hydroxide (17:2:1); Solvent B, chloroform–ethanol–28% ammonium hydroxide (15:20:1); Solvent C, chloroform–methanol–28% ammonium hydroxide (18:1:1); Solvent D, chloroform–acetone–diethylamine (5:4:1); Solvent E, ether–acetone–methanol–28% ammonium hydroxide (9:8:2:1); Solvent F, ether–methanol–28% ammonium hydroxide (17:2:1); Solvent G, chloroform–acetone–28% ammonium hydroxide (10:8:1); and Solvent H, methyl ethyl ketone–dimethylformamide–28% ammonium hydroxide (80:12:1).

Preparative separations were achieved using Solvent F with repeated developments for maximal separations as monitored under shortwave UV light. Analytically, the alkaloids were detected using sprays of dansyl chloride (12), tetrazotized benzidine (12), and a modified Pauly's reagent to react with imidazoles (16).

Resolution of Nonphenolic Alkaloids—Analytical TLC revealed that this fraction contained dolichotheline, a major unknown al-

[1] From The Cactus Gardens, Edinburg, Tex.; Davis Cactus Gardens, Kerrville, Tex.; and The Texas Cactus Ranch, Corpus Christi, Tex.

[2] A few characteristic plants were freeze dried and sent to Dr. Edward F. Anderson, Whitman College, Walla Walla, Wash., for confirmation of the identification. Living plants are being maintained as reference specimens in the greenhouse of the School of Pharmacy and Pharmacal Sciences, Purdue University.

[3] Ace all-glass circulating evaporator.

[4] Amberlite IRA 401S C.P. anion-exchange resin, Mallinckrodt Chemical Works.

[5] Baker-flex silica gel type IBF-2 and Brinkmann Silplate-52 glass plates.

[6] Brinkmann Instruments, Inc.

kaloid which fluoresced with dansyl chloride and produced a white chromophore with tetrazotized benzidine, and traces of five possible imidazoles that produced red chromophores with Pauly's reagent and gave R_f values in Solvent F at 0.29, 0.34, 0.43, 0.48, and 0.55. Ten milliliters from a 30-ml. ethanolic solution containing the total nonphenolic extract was condensed to a residue and, after four crystallizations from acetone–benzene (1:1), yielded a total of 1.338 g. of dolichotheline, melting point and mixed melting point 129–131° [lit. (1) m.p. 130–131°].

The mother liquor remaining from the dolichotheline crystallization was resolved *via* preparative TLC on 43 plates (20 × 20 cm.). The major unknown was eluted from the combined similar bands with ammoniacal ethanol (15). Analytical TLC of the condensed filtrates indicated chromatographic purity, and the hydrochloride was prepared by acidifying with anhydrous hydrogen chloride in absolute ethanol (5% w/w) and inducing crystallization by the dropwise addition of ether (yield 92.5 mg.).

Identification of Unknown Nonphenolic Alkaloid—A lime-green fluorescent dansyl conjugate of this alkaloid indicated a primary or secondary amine function (12). The IR spectrum[7] of the hydrochloride showed major bands at ν_{max}^{KBr} 2940, 2760 (broad, aromatic, and aliphatic C—H and salts), 1600, 1501, 1480 (aromatic C=C), 1315, 1080, 1055, 1020, 945, 890, 780, 740, and 690 cm.$^{-1}$. NMR spectra[8] showed peaks in deuterochloroform at δ 2.7 (singlet, 3H, CH_3—N), 3.2 (singlet, 4H, CH_2—CH_2), 7.3 (singlet, 5H, aromatic), and 9.8 (singlet, 1H, N—H). The low-resolution mass spectrum[9] showed a parent ion peak at *m/e* 135 (0.2%, $C_9H_{13}N$) and major fragmentation peaks at 91 (16.6%, C_7H_7), 65 (14.8%, C_5H_5), 44 (100%, CH_2NHCH_3), and 28 (14.2%, C_2H_4) consistent with those expected for phenethylamines.

Interpretation of these data suggested that the unknown alkaloid was simply *N*-methylphenethylamine hydrochloride. A reference sample of *N*-methylphenethylamine hydrochloride was synthesized from phenethylamine (17). Spectral data (IR, NMR, and mass spectrometry) obtained with the synthetic compound were essentially identical to those obtained with the isolated alkaloid hydrochloride. The melting points[10] and mixed melting points of the isolated and reference *N*-methylphenethylamine hydrochloride were the same (164–165°). Cochromatography of the isolate with the reference in five TLC systems (A, B, C, F, and G) substantiated this identification.

Resolution of Phenolic Alkaloids—Analytical TLC of this fraction revealed the presence of three major alkaloids and one minor alkaloid. Nine milliliters of a 26-ml. (total) ethanolic solution of this fraction was resolved *via* preparative TLC on 64 plates (20 × 20 cm.). The bands, after detection under UV light or by spraying the edge of the plate with tetrazotized benzidine, were removed and eluted. Further TLC analysis of the condensed eluates allowed a tentative identification of three alkaloids as *N*-methyltyramine, *β-O*-methylsynephrine, and synephrine. Crystalline hydrochlorides for the first two were then prepared as described previously.

Identification of Phenolic Alkaloids—Cochromatography with reference racemic *β-O*-methylsynephrine hydrochloride in five solvent systems (A, C, F, G, and H) tentatively identified this compound as one of the crystallized phenolic alkaloids. Melting points and mixed melting points were similar (186–189°). IR spectral data were comparable between the isolated and reference racemic *β-O*-methylsynephrine hydrochloride; however, slight shifts and different intensities of a few bands in the IR spectrum of the isolate when compared to that of the racemic reference suggested that the isolate was likely (−)-*β-O*-methylsynephrine hydrochloride (15, 18). Determination of the optical rotation was not attempted due to the low yield (13.5 mg.). The NMR spectra of the two compounds in deuterated methanol compared identically, with peaks at δ 2.6 (singlet, 3H, CH_3—N), 3.2 (multiplet, 5H, containing a singlet, 3H, CH_3—O, and a doublet, 2H, CH_2—C—H), 4.3 (weak multiplet, 1H, benzylic), and 7.0 (quartet, 4H, *para*-substituted aromatic). Mass spectral analysis showed parent ion peaks at *m/e* 181 and the expected fragmentation.

Reference *N*-methyltyramine hydrochloride and the second crystalline hydrochloride consistently cochromatographed (Solvents A, B, C, F, and G). The melting point of the isolate (146–148°) and mixed melting point (147–150°) with reference *N*-methyltyramine hydrochloride proved to be similar. The IR spectra were essentially identical, and both showed bands at ν_{max}^{KBr} 3260 (phenolic OH), 2890, 2810 (broad, aromatic and aliphatic C—H, secondary amine salt), 1620, 1600, 1530 (aromatic C=C), 1220 (C—O), and 820 cm.$^{-1}$. The NMR spectra showed peaks in deuterium oxide at δ 2.8 (singlet, 3H, N—CH_3), 3.1 (multiplet, 4H, containing two overlapping triplets, CH_2—CH_2), and 7.1 (quartet, 4H, *para*-substituted aromatic). Mass spectral analysis of the isolate showed a parent ion peak at *m/e* 151 and major fragmentation peaks corresponding to the fragmentation observed with reference *N*-methyltyramine hydrochloride.

The eluates containing synephrine were further separated *via* preparative TLC on eight plates (20 × 20 cm.) to yield synephrine and an unknown alkaloid which cochromatographed with the unknown minor phenolic alkaloid previously mentioned. These latter eluates were combined, and the hydrochlorides of these compounds were then successfully crystallized.

Cochromatography of the isolated synephrine hydrochloride and reference synephrine hydrochloride in Solvents A, B, C, F, and G substantiated the identification of the isolate as synephrine. Melting points and mixed melting points of the isolate and reference racemic synephrine hydrochloride were similar (153–155°). The IR spectra of the isolate and reference racemic material were similar but exhibited some differences (15, 18); however, the IR spectrum of the isolate and that of natural (−)-synephrine hydrochloride (15) were essentially identical, and the isolate is assumed to be (−)-synephrine hydrochloride. The NMR spectrum in deuterium oxide showed peaks at δ 2.6 (singlet, 3H, N—CH_3), 3.2 (doublet, 2H, CH_2—C—H), 4.9 (multiplet overlapping D_2O peak, 1H, benzylic), and 7.1 (quartet, 4H, *para*-substituted aromatic). Mass spectral analysis showed a parent ion peak at *m/e* 167 and major fragmentation peaks consistent with those observed for reference synephrine hydrochloride.

The IR spectrum of the unknown phenolic alkaloid showed bands at ν_{max}^{KBr} 3240, 2980 (broad, aromatic and aliphatic C—H, secondary amine salts), 1620, 1610, 1600, (aromatic C=C), 1300, and 1110 (ether, aryl C—O) cm.$^{-1}$. Due to the small amount of material isolated (8.8 mg.), an NMR spectrum was not obtained. Analysis of high-[11] and low-resolution mass spectral data allowed the assignment of *m/e* 195 (2.3%, $C_{11}H_{17}NO_2$) as the parent ion. The major fragmentation peaks that aided in the structural assignment were at 151 (100%, $C_9H_{11}O_2$), 150 (18.6%, $C_9H_{12}NO$), 123 (82.5%, $C_7H_7O_2$), and 77 (24.4%, C_6H_5). The UV spectrum[12] showed two peaks at 225.5 and 275 nm., with $E_{1\,cm.}^{1\%}$ values equal to 450 and 75, respectively; in base these peaks showed a bathochromic shift typical of *para*-hydroxyphenols. Interpretation of these data suggested that the unknown was *β-O*-ethylsynephrine.

Synthesis of Reference *β-O*-Ethylsynephrine—A modification of the procedure of Stewart and Wheaton (18) was used. One gram of synephrine base[13] was added to an excess (15 g.) of thionyl chloride under nitrogen. After a few minutes, the precipitate was washed with benzene and again with ether. Ten milliliters of ethanol was added, and the mixture was refluxed in a water bath for 1 hr. The resulting solution was condensed to a small volume, and the addition of acetone induced crystallization. Treatment with activated charcoal and recrystallization from ethanol and ether yielded 512 mg. (51% yield) of synthetic (±)-*β-O*-ethylsynephrine hydrochloride (m.p. 169–171°). IR, NMR, and mass spectral data identified the material as the desired synthetic product.

Identification of Unknown Phenolic Alkaloid as *β-O*-Ethylsynephrine—TLC comparison of the synthetic *β-O*-ethylsynephrine hydrochloride with the isolated unknown alkaloid resulted in cochromatography (Solvent F) and identical chromophore formation with tetrazotized benzidine. Melting points of the isolated alkaloid (167–169°) and mixed melting points (170–173°) further supported this identification. IR and mass spectral data were likewise confirmatory.

To substantiate the natural occurrence of this compound in the plant, a new extraction of a 20-g. sample of *D. sphaerica* was made using methanol in place of ethanol and deleting ether in the original

[7] Beckman 33 IR spectrophotometer.
[8] Jeol MH-II NMR spectrometer using a microtube.
[9] Hitachi RMU-6A mass spectrometer.
[10] Determined with a Mel-Temp apparatus, corrected.
[11] Obtained on a CEC 21-110 at 70 ev. at Hoffmann-La Roche, Inc.
[12] Cary model 17 UV spectrophotometer.
[13] Sigma Chemical Co.

Table I—Quantities of Alkaloids Isolated from *D. sphaerica*

Alkaloid	Yield, mg.	Concentration in Cactus, % w/w
Dolichotheline	1338.0	0.6500
N-Methylphenethylamine hydrochloride	92.5	0.0411
β-*O*-Methylsynephrine hydrochloride	13.5	0.0060
N-Methyltyramine hydrochloride	26.0	0.0115
Synephrine hydrochloride	7.5	0.0033
β-*O*-Ethylsynephrine	8.8	0.0038

extraction procedure. Following this new extraction, analytical TLC (Solvent F) of Fractions A and C showed the complete absence of this alkaloid in the extracts. Apparently, since several steps in the usual extraction procedure utilize acid and ethanol, there was an ethylation of some natural synephrine to produce β-*O*-ethylsynephrine as an artifact.

DISCUSSION

As summarized in Table I, six alkaloids were isolated as their hydrochlorides from alkaloid extracts of *D. sphaerica*. Identifications of the compounds were achieved by comparing data (IR, NMR, and mass spectral), TLC behavior, and melting points with those of reference alkaloids. The isolated alkaloids were dolichotheline, as expected from previous reports (1, 2), *N*-methylphenethylamine hydrochloride, *N*-methyltyramine hydrochloride, synephrine hydrochloride, β-*O*-methylsynephrine hydrochloride, and β-*O*-ethylsynephrine hydrochloride. Since ethyl groups are rare in nature, reextraction of additional plant material, taking care to eliminate any contact with ethyl-containing compounds, was performed; TLC analysis then failed to detect β-*O*-ethylsynephrine in the new extracts. Apparently, this compound was isolated as an extraction artifact of synephrine; similar *O*-alkylation of synephrine while extracting it from tangerine leaves was reported (18).

N-Methylphenethylamine, the major new alkaloid isolated, has never before been isolated from the Cactaceae. This compound was first isolated in 1939 from *Arthrophytum leptocladum* Popov in the Chenopodiaceae (19, 20) and has since been isolated from several *Acacia* species (Leguminosae) (21–26). Of particular interest is its isolation from *Acacia berlandieri* Benth. as a major toxic constituent (26); goats and sheep in west Texas eat this shrub in large amounts during prolonged drought and suffer from a condition known as "Limberleg" or "Guajillo Wobbles." However, the acute action of *N*-methylphenethylamine as a pressor agent has been shown to occur experimentally with low toxicity (27, 28). Logical biosynthetic precursors for this alkaloid would be phenylalanine and methionine, following a similar two-step pathway as in the formation of *N*-methyltyramine from tyrosine in *Hordeum* (29).

N-Methyltyramine was first isolated from *Hordeum* in 1950 (17) and has previously been identified in several cactus species as well as other plant families (12, 13, 15, 30–33). It possesses weak sympathomimetic activity (34, 35).

β-*O*-Methylsynephrine has been isolated twice previously from the Cactaceae (15, 36) and from citrus leaf extracts as an artifact of synephrine (18). Comparison of IR spectra of the isolated and racemic reference β-*O*-methylsynephrine hydrochloride suggested that the isolate is the (−)-isomer. If this compound were made from synephrine during the extraction procedure, the racemic mixture would have been isolated. No reported studies concerning the pharmacological action of β-*O*-methylsynephrine were found; however, a similar compound, β-*O*-methylepinephrine, produces considerable central nervous system stimulation (37).

Synephrine was previously isolated and detected in *Citrus* (38), and it was detected in three other plant families (33) as well as in human urine (39). Recently, it was detected and isolated in several cacti (15, 36, 40, 41). Synephrine is a well-known sympathomimetic agent (42).

While the pharmacological action of the most abundant alkaloid, dolichotheline, remains unknown, the presence of these sympathomimetic β-phenethylamine alkaloids confers explainable physiological activity on this cactus and, perhaps, helps to explain its folkloric connections with *Lophophora*.

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ACKNOWLEDGMENTS AND ADDRESSES

Received April 20, 1973, from the *Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907*

Accepted for publication June 1, 1973.

Presented to the Pharmacognosy and Natural Products Section,

APHA Academy of Pharmaceutical Sciences meeting with the American Society of Pharmacognosy, Jekyll Island, Georgia, July 1973.

Supported in part by U. S. Public Health Service Grant MH-21448-01 from the National Institute of Mental Health, General Research Support Grant RRO-5586 from the National Institutes of Health, and a grant through the Purdue University Cancer Research Committee from the Indiana Elks.

The authors thank Dr. H. Rosenberg, University of Nebraska, for samples of dolichotheline and for suggesting this project; Dr. E. F. Anderson, Whitman College, for confirming the plant identification; Dr. G. E. Peck, Purdue University, for assistance with the Fitzpatrick mill; and Dr. W. Benz, Hoffmann-La Roche, Inc., for determining and interpreting high-resolution mass spectra.

▲ To whom inquiries should be directed.

Kinetics and Mechanisms of Drug Action on Microorganisms XVII: Bactericidal Effects of Penicillin, Kanamycin, and Rifampin with and without Organism Pretreatment with Bacteriostatic Chloramphenicol, Tetracycline, and Novobiocin

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Abstract □ *Escherichia coli* (ATCC 12407), generating in the logarithmic growth phase, was killed by penicillin, kanamycin, and rifampin. The logarithm of number of viables of the drug-treated culture decreased linearly with time after a certain lag period and above a minimum drug concentration. The slopes of these plots were characteristic of kill rate constants and were linearly dependent on drug concentrations. The microorganisms developed resistance against the cidal action of the antibiotics. The lower the penicillin concentration and the rate of kill, the greater is the number of resistant organisms, most probably formed by generation in the presence of penicillin. Penicillin was inactivated with time in the culture of organisms, and the resistant individuals eventually grew and were shown to be insensitive to penicillin. When organisms were exposed to the cidal action of kanamycin or rifampin, the appearance of resistant individuals was ascertained. Penicillin yielded debris and ghost cells of *E. coli*. No such apparent lysis was observed with kanamycin or rifampin. The activity of kanamycin increased with the pH of the medium, so activity can be assigned to the uncharged or lesser charged fraction of the drug concentration. The addition of penicillin or kanamycin to the organisms treated with tetracycline, chloramphenicol, or novobiocin did not show any significant difference in killing rate from organisms not previously treated with such bacteriostatic agents. However, the combination of a bactericidal and a bacteriostatic antibiotic depressed the resistant mutant formation of resistant individuals over that of the bactericidal drug alone. When rifampin was added to organisms pretreated with tetracycline or chloramphenicol, the cidal action of rifampin was significantly reduced.

Keyphrases □ Bactericidal activity, kinetics and mechanisms—penicillin, kanamycin, and rifampin with and without *E. coli* pretreatment with chloramphenicol, tetracycline, or novobiocin □ Antibiotics, kinetics and mechanisms of bactericidal activity—penicillin, kanamycin, and rifampin with and without *E. coli* pretreatment with chloramphenicol, tetracycline, or novobiocin □ Bacteriostatic *E. coli* pretreatment with chloramphenicol, tetracycline, or novobiocin—effect on bactericidal activity of penicillin, kanamycin, and rifampin

Antibacterial agents have two possible modes of action: inhibition of microbial generation and/or "kill" superimposed on generation. They can be differentiated by concomitantly monitoring the microorganism concentration of the nutrient medium containing the agent by viable (colony) counts and total (Coulter) counts as a function of time. It was shown (1) that the total counts and colony counts are coincident for the bacteriostatic tetracyclines, chloramphenicol, sulfonamides, macrolides, lincosaminides, *etc.*, in the concentration ranges below the amount resulting in complete inhibition of microbial generation.

The bactericidal action of drugs in defined concentration ranges must be kinetically defined by the time-consuming and laborious colony count method. This article reports on studies designed to quantify systematically the effects on microbial generation of several bactericidal antibiotics: penicillin, kanamycin, and rifampin.

The primary action of penicillin is in the inhibition of the production of cell wall material, specifically in the biosynthesis of murein (2). Presumably by interacting with the acceptor site of the ribosome, kanamycin may cause significant conformational changes in ribosomes which induce ambiguity in the readout of mRNA. (2). Rifampin was shown (2) to inhibit DNA-dependent RNA synthesis by specifically interfering with the function of the RNA polymerase by forming a stable rifampin–polymerase complex.

EXPERIMENTAL

Microorganism—Replicate slants of *Escherichia coli* (ATCC 12407) were used in all experiments.

Culture Media—Bacto Antibiotic Medium 3[1], rehydrated (pH 7.05) according to the specifications of the manufacturer to Medium 3 USP, was used. The culture medium was filtered through a 0.45-μ filter[2] and autoclaved at 120° for 15 min. To obtain media in the pH range of 6.30–7.55, various amounts of concentrated hydrochloric acid and sodium hydroxide solution were added aseptically to the culture media.

Antibiotic—The assayed samples of sodium penicillin G[3] (1625

[1] Difco Laboratories Detroit, Mich.
[2] Millipore HA.
[3] Obtained from The Upjohn Co., Kalamazoo, Mich.