SYNTHESIS OF SPECIFICALLY ^2H-LABELED RESERPINES, 3,4,5-TRIMETHOXYBENZOIC ACIDS, AND SYRINGIC ACID

Robert W. Roth,* Daniel L. Fischer,* Janet M. Pachta,* and James F. Althaus**

*BioOrganic Chemistry Department, Midwest Research Institute
425 Volker Boulevard, Kansas City, Missouri 64110

**Division of Chemistry, National Center for Toxicological Research
Jefferson, Arkansas 72079

SUMMARY

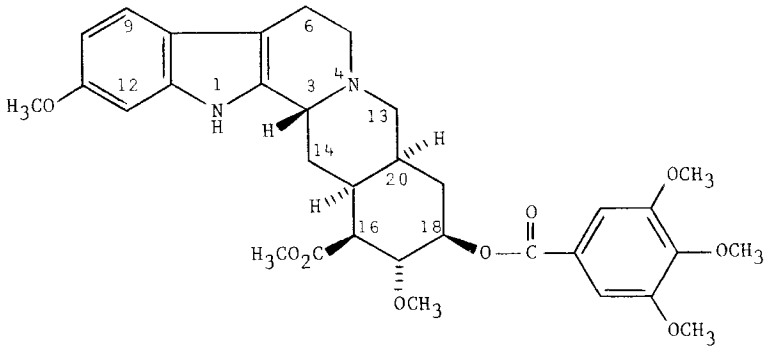
3,4,5-Trimethoxy-$^2H_9$-, 4-methoxy-3,5-dimethoxy-$^2H_6$-, and 4-hydroxy-3,5-dimethoxy-$^2H_6$-benzoic acids were prepared from n-propyl 3,4,5-trihydroxybenzoate (n-propyl gallate) by means of appropriate alkylation-hydrolysis sequences employing iodomethane-$^2H_3$ or dimethyl-$^2H_6$-sulfate as the deuterium source. 4-Methoxy-$^2H_3$-3,5-dimethoxybenzoic acid was similarly prepared from ethyl 4-hydroxy-3,5-dimethoxybenzoate. The labeled trimethoxybenzoic acids were converted to the corresponding ^2H-labeled reserpines by condensation of the acid chlorides with methyl reserpate in pyridine according to the classical procedure. The labeled reserpine analog methyl 18-O-(4-hydroxy-3,5-dimethoxy-$^2H_6$)benzoyl reserpate was likewise prepared from 4-hydroxy-3,5-dimethoxy-$^2H_6$-benzoic acid via the intermediate methyl 18-O-(4-ethoxycarbonyloxy-3,5-dimethoxy-$^2H_6$-benzoyl)reserpate (syrosingopine-$^2H_6$). The isotopic purity of each compound exceeded 99 atom percent ^2H.

Key Words: 3,4,5-Trimethoxy-$^2H_9$-benzoic acid, 4-Methoxy-3,5-dimethoxy-$^2H_6$-benzoic acid, 4-Methoxy-$^2H_3$-3,5-dimethoxybenzoic acid, Syringic-$^2H_6$ acid, Deuterated reserpines

INTRODUCTION

Since its introduction in the late 1950's, the antihypertensive reserpine (11,17α-dimethoxy-18β-[(3,4,5-trimethoxybenzoyl)oxy]-3β-20α-yohimban-16β-carboxylic acid methyl ester or methyl 18-O-(3,4,5-trimethoxybenzoyl)reserpate) (1) has become one of the most widely dispensed drugs in modern pharmacopoeias. Studies comparing bioavailability and bioequivalence of various commercial formulations, which contain only 0.1 to 1.0 mg of the free base, require sensitive analytical techniques for quantifying levels of the drug or its metabolites in the bloodstream. Presently accepted methods involve dissolution studies, spectrophotometric assays, and thin-layer chromatography [1,2]. Recent reports [3-9] linking reserpine administration to breast cancer in humans have given impetus to the development of even more sensitive methods of detecting and measuring the drug in

0362-4803/82/080963-11$01.10 Received April 1, 1982



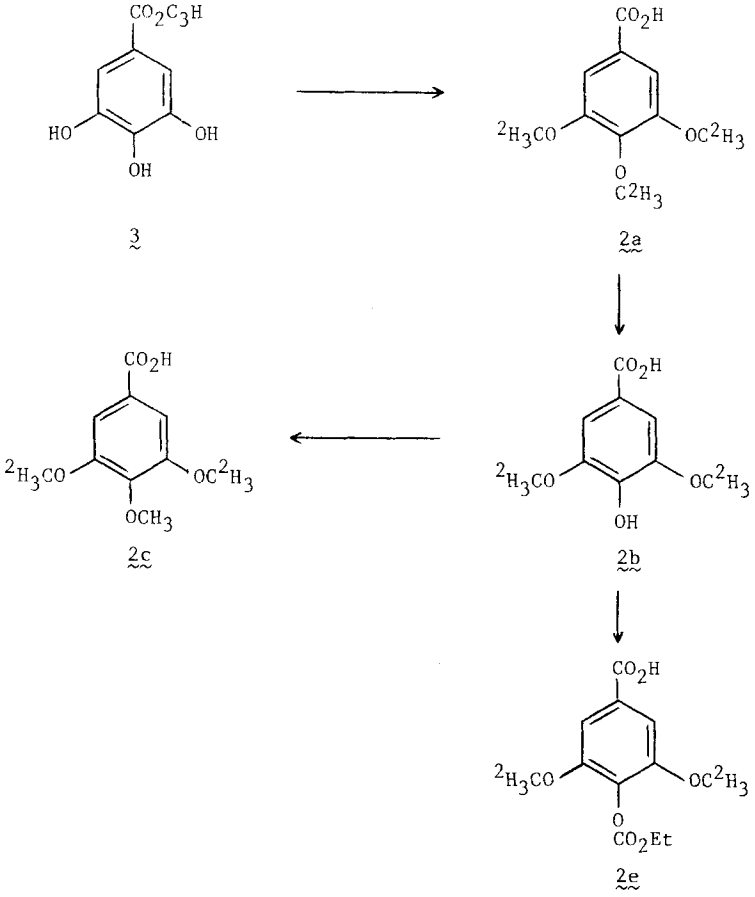
1

biological systems. The inherent sensitivity of mass spectrometry combined with the excellent precision of stable isotope dilution techniques makes this method quite attractive. To this end we have prepared several deuterium-labeled analogs of reserpine, choosing the relatively accessible methyl groups of the trimethoxybenzoyl substituent as the sites of label incorporaton. A particular advantage of this approach lies in its potential extension to deuterium- and tritium-labeled analogs of numerous other CNS active drugs which contain a 3,4,5-trimethoxybenzoyl moiety.

RESULTS AND DISCUSSION

The syntheses of 3,4,5-trihydroxybenzoic acid (gallic acid) methyl-$^2H_3$ ethers 2a through 2e are outlined in Scheme 1. Alkylation of n-propyl gallate (3) with 3 mole equivalents of dimethyl-$^2H_6$ sulfate in refluxing 2-butanone in the presence of potassium carbonate or with 3.3 mole equivalents of iodomethane-$^2H_3$ in dimethylformamide containing potassium hydroxide at room temperature afforded 3,4,5-trimethoxy-$^2H_9$-benzoic acid (2a) in high yield. Demethylation of 2a in warm concentrated sulfuric acid [10] afforded 4-hydroxy-3,5-dimethoxy-$^2H_6$-benzoic acid (syringic-$^2H_6$ acid, 2b) in good yield and undiminished isotopic purity. This method of hydrolysis does, however, give syringic acid initially contaminated with significant amounts of starting material as well as unidentified

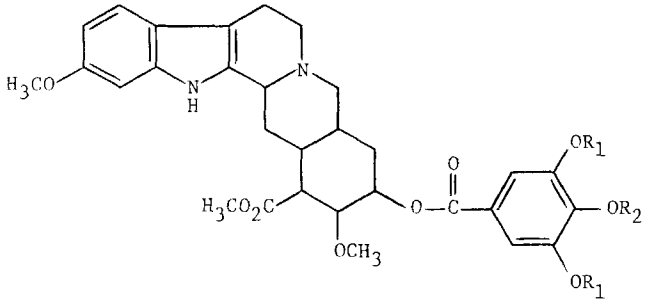
Scheme 1


CO2C3H
HO
OH
OH
3
CO2H
2H3CO
OC2H3
O
C2H3
2a
CO2H
2H3CO
OC2H3
OH
2b
CO2H
2H3CO
OC2H3
OCH3
2c
CO2H
2H3CO
OC2H3
O
CO2Et
2e
CO2Et
H3CO
OCH3
OH
4
CO2H
H3CO
OCH3
O
C2H3
2d

mono- and dimethyl ethers of gallic acid, all of which would be converted to trimethoxybenzoic acid esters upon treatment with dimethyl sulfate. Therefore, the purity of 2b was confirmed by HPLC analysis prior to its conversion to 4-methoxy-3,5-dimethoxy-$^2H_6$-benzoic acid (2c), thus assuring maximum isotopic purity and label specificity in the latter compound. Alkylation of 2b and 4 proceeded readily using dimethyl sulfate/K_2CO_3 in 2-butanone. However, syringic acid failed to react efficiently with iodomethane/KOH in DMF beyond formation of methyl syringate. It seems reasonable to conclude, therefore, that methylation of n-propyl gallate under these conditions does not involve propyl syringate as a principal intermediate.

The 70 ev mass spectra of 2a through 2d indicated an isotopic purity near 99.5 atom percent ^2H for each compound. The ^1H NMR spectra in $CDCl_3$ solution of methyl-deuterated 3,4,5-trimethoxybenzoic acids and methyl or propyl esters are useful in determining only the total deuterium content since all three methyl groups have coincident chemical shifts [11].

The conversion of the labeled trimethoxybenzoic acids and ethoxycarbonylsyringic acid (2e) [12] to reserpine analogs 1a through 1e was accomplished by condensation of the corresponding acid chlorides with methyl reserpate in pyridine solution following previously described procedures [13,14]. HPLC analysis indicated a chemical purity of > 98% for each compound. Although the mass



1a $R_1, R_2 = C^2H_3$
1b $R_1 = C^2H_3, R_2 = H$
1c $R_1 = C^2H_3, R_2 = CH_3$
1d $R_1 = CH_3, R_2 = C^2H_3$
1e $R_1 = C^2H_3, R_2 = CO_2Et$

spectrum of reserpine at 20-70 ev contains a major fragment at m-1 and smaller fragments at m-2 and m-3 which preclude an accurate determination of deuterium content for the labeled analogs, the mass spectra of 1a, c, and d were consistent with an isotopic purity of > 99 atom percent ^2H. The mass spectrum of 1b, which is free of fragments to m-15, indicated an isotopic purity of 99.6 atom percent ^2H.

EXPERIMENTAL

All melting points are uncorrected. Mass spectra were obtained by direct inlet using Varian/MAT CH-4, CH-5, or Finnigan 1015 spectrometers. NMR spectra were recorded on a Varian EM360 instrument in $CDCl_3$ solution using tetramethylsilane as an internal standard. Ultraviolet spectra were recorded on a Varian Superscan 3 spectrometer at a spectral band width of 1 nm. Thin-layer chromatography was performed on E. Merck Silica Gel 60 analytical plates unless specified otherwise. HPLC analyses were carried out using one of the following systems: System 1: 250 x 4.6 mm ID 5 μ ODS Spherisorb column, CH_3CN:H_2O:HOAc (50:50:1) at 1 ml/min, detection at 286 nm. System 2: 250 x 4.6 mm ID Partisil-10 ODS-2 column, CH_3CN:0.1% HOAc in H_2O (25:75 to 50:50 10-min linear program) at 1.4 ml/min, detection at 280 nm. System 3: 300 x 3.9 mm ID μBondapak C_{18} column, CH_3OH:H_2O:HOAc (54:46:1) at 1 ml/min, detection at 254 nm. System 4: identical to System 3 except CH_3OH:H_2O:HOAc (50:50:1). Dimethyl-$^2H_6$ sulfate and iodomethane-$^2H_3$, 99+ atom percent ^2H, were purchased from Aldrich Chemical Company.

3,4,5-Trimethoxy-$^2H_9$-benzoic Acid (2a)

Dimethyl sulfate method: N-Propyl gallate (10 g, 47 mmol) was dissolved in 125 ml of 2-butanone and heated at reflux. A total of 20 g (152 mmol) of dimethyl-$^2H_6$ sulfate and 20 g (145 mmol) of anhydrous K_2CO_3 was added in small portions over 24 hr. HPLC analysis (System 1) was performed periodically, and when the gallate appeared to be less than 1% of the product (28 hr), the mixture was filtered, the potassium salts washed with acetone, and the filtrate evaporated to dryness. The residue was taken up in a minimum volume of methanol and added to

100 ml of H_2O containing 10 g of KOH. The mixture was heated at 100°C for 1 hr, acidified while hot with concentrated HCl, and allowed to cool slowly. The hot solution deposited a mass of fine, white needles which were filtered cold and washed free of salts with cold H_2O. The yield was practically quantitative, m.p. 165-167°C (lit. [10] 168°C). The 70 ev mass spectrum indicated an isotopic purity > 99 atom percent ^2H.

Iodomethane method: Potassium hydroxide (11.5 g, 175 mmol, 85% pellets) was added to a well-stirred solution of n-propyl gallate (11.17 g, 52.6 mmol) in 75 ml of dimethylformamide at room temperature. After 10 min, 10 g of iodomethane-$^2H_3$ was added, producing a mildly exothermic reaction which was moderated by external coolng with a 25°C water bath. An additional 15 g (25 g, 172 mmol total) of iodomethane-$^2H_3$ was then added in three equal portions at ca. 10-min intervals and reaction progress monitored by TLC on Eastman Chromogram (silica gel) strips developed in benzene. The progression from mono- to trimethoxy ester (highest R_f component) was complete within 30 min of the final addition. The reaction mixture was poured into 500 ml of water, the solution made strongly basic with 6 N NaOH, and extracted with 3 x 175 ml of benzene. The extracts were washed with water, dried (Na_2SO_4), and evaporated in vacuo to give 11.6 g (84%) of the trimethoxy-$^2H_9$ ester as a colorless oil. A 10.4 g sample of crude ester afforded 8.55 g (97.5%) of the corresponding acid, m.p. 165-168°C, > 99 atom percent ^2H, upon hydrolysis as described above.

4-Hydroxy-3,5-dimethoxy-$^2H_6$-benzoic Acid (Syringic-$^2H_6$ Acid, 2b)

A mixture of 5.748 g (26.0 mmol) of trimethoxy-$^2H_9$-benzoic acid and 29 ml of concentrated sulfuric acid was heated in a 40°C water bath for 7 hr, then cooled and maintained at room temperature for an additional 20 hr. The resulting purple solution was poured with vigorous stirring into 28.5 ml of water, and upon cooling to room temperature, 3.417 g of white needles, m.p. 201-203.5°C, ∿ 95% pure by HPLC analysis (System 3), was obtained. The product was further purified by dissolving in a mixture of 75 ml of benzene and 50 ml of acetone, concentrating the hot solution to a volume of 90 ml under a stream of nitrogen and cooling.

The resulting product was recrystallized again in the same manner to give 2.239 g of the title compound, m.p. 203-207°C (lit. [10] 204-206.5°C), > 99% purity by HPLC analysis (System 2, retention time 12 min). The aqueous filtrate from the initial isolation was concentrated in vacuo to provide additional material, which was combined with that recovered from the above mother liquors. Following recrystallization, once from water and twice from benzene-acetone, an additional 0.935 g of product, 99% purity, was obtained (combined yield, 60%). The 20 ev mass spectrum of the product indicated an isotopic purity of 99.6 atom percent ^{2}H.

4-Methoxy-3,5-dimethoxy-$^2H_6$-benzoic Acid (2c)

A solution of 5.10 g (25.0 mmol) of syringic-$^2H_6$ acid in 65 ml of 2-butanone was heated to reflux and treated with a total of 8.0 ml (10.7 g, 84.6 mmol) of dimethyl sulfate and 10.4 g (75.3 mmol) of anhydrous K_2CO_3, each added in four equal portions over 6 hr. Reaction progress was monitored by TLC (ether-hexanes-acetic acid, 66:33:1), which showed an initial rapid conversion to methyl syringate-$^2H_6$ followed by a gradual transformation to the trimethoxy ester which was complete after overnight reflux. Isolation and hydrolysis as described for 2a afforded 5.34 g (98%) of the title compound, m.p. 168-170°C. The 70 ev mass spectrum indicated an isotopic purity of 99.6 atom percent ^{2}H.

Ethyl Syringate (4)

A mixture of 20 g (101 mmol) of syringic acid in 300 ml of absolute ethanol containing 1 ml of concentrated HCl and 0.1 ml of trifluoroacetic acid was heated to distill one-half the solvent, which was replaced by an equal volume of 1:1 benzene-ethanol. This process was repeated until HPLC analysis (System 1) indicated > 95% conversion to the ester, following which the solution was cooled and evaporated to dryness in vacuo. The residue was taken up in 200 ml of benzene, washed with 3 x 100 ml of saturated aqueous $NaHCO_3$ containing 250 mg of K_2CO_3, dried (Na_2SO_4), and evaporated to dryness. Recrystallization from benzene-ethanol (95:5) gave large rhombohedra, m.p. 85-86°C.

4-Methoxy-$^2H_3$-3,5-dimethoxybenzoic Acid (2d)

This compound (m.p. 166-167°C) was prepared in nearly quantitative yield from ethyl syringate (10 g) following essentially the same procedure as described for 2a using dimethyl-$^2H_6$ sulfate (5.0 g), K_2CO_3 (5.0 g), 2-butanone (100 ml), and a total reaction time of 20 hr for the alkylaton step. The mass spectrum indicated an isotopic purity > 99 atom percent ^2H.

4-Ethoxycarbonyloxy-3,5-dimethoxy-$^2H_6$-benzoic Acid (2e)

To 2.200 g (10.8 mmol) of syringic-$^2H_6$ acid dissolved in 25 ml of 1 N NaOH at 0°C was added 2.0 ml (2.27 g, 20.9 mmol) of ethyl chloroformate. The mixture was stirred overnight at room temperature under N_2, cooled, and adjusted to pH 2 with 5% HCl. Water (50 ml) was then added and the product collected and washed with water. Recrystallization from 35 ml of methanol gave 2.435 g (82%) of the title compound as white prisms, m.p. 182-186°C (lit. [12] 178-181°C).

Deuterium-labeled Reserpines 1a, 1c, and 1d

The general method is illustrated by the procedure for 1a. Methyl reserpate [m.p., 239-241°C (242-245°C, evacuated tube), lit. [13] 241-249°C] was prepared in 60% yield by hydrolysis of reserpine in methanolic KOH essentially as described by Dorfman [13]. Dissolution of the crude product in hot tetrahydrofuran and crystallization at -20°C was found to be superior to the described recrystallization from methanol.

A mixture of 2.044 g (9.25 mmol) of 3,4,5-trimethoxy-$^2H_9$-benzoic acid and 2.5 ml of thionyl chloride in 10 ml of benzene was heated to reflux for 4 hr, during which the acid dissolved to produce a homogeneous solution. An aliquot quenched in methanol and examined by TLC confirmed that conversion to the acid chloride was complete. Benzene and excess thionyl chloride were removed *in vacuo*, leaving the white crystalline acid chloride which required no further purification.

Methyl reserpate (3.83 g, 9.25 mmol) was dissolved in 30 ml of pyridine (previously dried over 4A molecular sieve) and added dropwise over 10 min with

stirring to the solid acid chloride cooled in an ice bath. The mixture was stirred at 0°C for 1.5 hr, then warmed over 1.5 hr to room temperature and stirred for an additional 1 hr. The dark yellow reaction mixture was then added gradually with vigorous stirring to 600 ml of ice cold water to precipitate the product as a very finely divided white solid which gradually coagulated and was collected, washed with water, and dried in vacuo. The crude product was dissolved in 30 ml of methylene chloride and chromatographed on a 4 x 8 cm column of silica gel, eluting with methylene chloride-methanol (95:5). Reserpine eluted in the first 500-ml fraction, which was concentrated in vacuo to approximtely 30 ml and added gradually to 100 ml of boiling methanol. Boiling was continued until the temperature of the distillate reached 62°C and much of the product had crystallized. Upon cooling (0°C), 3.47 g of the title compound was obtained. The yield after a second recrystallization by the same procedure was 3.396 g (89%), m.p. 290-291°C, d. (evacuated tube), (lit. [13] 277-277.5°C).

HPLC analyses of 1a, c, and d (System 3, retention time 9.4 min) indicated a purity > 99%. The UV spectra in 95% ethanol [λ(ε): 264 (16630), 291 (10410)] were identical to that of unlabeled reserpine. The 60 MHz ^{1}H-NMR spectra of 1a, c, and d in $CDCl_3$ were identical to that of reserpine except for the singlet at δ 3.9 which integrated for 0, 3, and 6 protons respectively (9 protons in reserpine). The 70 ev EI mass spectra contained the respective molecular ions (base peak) at m/z 617, 614, and 611, characteristic fragment ions corresponding to M-1, M-15, and M-18, an intense ion at m/z 395, and trimethoxybenzoyl ions at m/e 204, 201, or 198. The IR spectra (nujol mull) contained C-D stretching bands in the 2060-2220 cm^{-1} region but were otherwise identical to the spectrum of the parent compound.

Methyl 18-O-(4-Hydroxy-3,5-dimethoxy-$^{2}H_6$-benzoyl)reserpate (1b)

Ethyl chloroformate (2.0 ml, 21 mmol) was added dropwise to a solution of 2b (2.2 g, 10.8 mmol) in 1 N NaOH at 0°C. The mixture was stirred overnight at room temperature, then cooled and adjusted to pH 2 with 5% HCl. Water (50 ml) was added and the product collected by suction filtration. Recrystallization from

methanol (35 ml) gave 2.44 g (82%) of 4-ethoxycarbonyloxy-3,5-dimethoxy-$^2H_6$-benzoic acid (2e) as white prisms, m.p. 182-186°C (lit. [12] m.p. 178-181°C). The corresponding acid chloride was obtained by reaction with excess thionyl chloride in benzene as described for the trimethoxybenzoic acids above. A solution of methyl reserpate (3.8 g, 9.2 mmol) in 20 ml of dry pyridine was added to a stirred solution of the crude acid chloride in 10 ml of pyridine at 0°C. The mixture was stirred at room temperature for 2 hr, then added gradually to 1,200 ml of water to precipitate the product (3.44 g). The crude ethoxycarbonyl ester was suspended in a warm mixture of ethanol (40 ml) and concentrated NH_4OH (40 ml) for 30 min, during which the starting material dissolved and the product precipitated. The volume of the reaction mixture was reduced to 25 ml under reduced pressure, and the product was collected and recrystallized twice from methanol to give 2.08 g (38%) of the title compound, m.p. 198-202°C with decomposition, m.p. (evacuated tube) 245-246°C (lit. [14] 190-192°C). TLC: methylene chloride: acetone:ethyl acetate (2:1:1), R_f = 0.3. HPLC analysis (System 4, retention time 7.4 min) indicated a chemical purity ≥ 98.6%. The NMR spectrum was identical to that of an unlabeled standard except for the absence of a 6 proton singlet at δ 3.9. The UV spectrum [95% ethanol, λ(ε): 2.74 (17180), 288 (16720)] was also identical to that of the unlabeled compound. The 70 ev mass spectrum contained a molecular ion at m/z 600 (base peak) and one fragment (m/z 395) having a relative intensity > 15% of the base peak.

ACKNOWLEDGMENT

This work was supported in part by the Food and Drug Administration, Contracts Nos. 222-76-2037(C) and 222-80-2002(C).

REFERENCES

1. Tripp S.L., Williams E., Wagner W.E. and Lukas G. - Life Sciences 16: 1167 (1975).

2. Sams R.A. and Hoffman R. - J. Chrom. 161: 410 (1978).

3. Smitthline F., Sherman L. and Kolodny H.D. - New Eng. J. Med. 292: 784 (1975).

4. Boston Collaborative Drug Surveillance Program - Lancet 2: 292 (1975).

5. Mack T.M., Henderson B.E., Gerkins V.R., Baptista J. and Pike M.C. - New Eng. J. Med. 292: 1366 (1975).

6. Heinonen D.P., Shapiro S., Tuominen L. and Turunen M.I. - Lancet 2: 675 (1974).

7. O'Fallon W.M., Labarthe D.R. and Kurland L.T. - Lancet 2: 292 (1975).

8. Schoental R. - Lancet 2: 1571 (1974).

9. Hadles H.I. - Lancet 1: 169 (1975).

10. Bogert M.T. and Coyne B.B. - J. Amer. Chem. Soc. 51: 569 (1929).

11. The Aldrich Library of NMR Spectra, Vol. 6, p. 176.

12. Chodnekar M.S., Sharp L.K. and Linnell W.H. - J. Pharm. Pharmacol. 14: 756 (1962).

13. Dorfman L., Furlenmeier A., Huebner C.F., Lucas R., MacPhillamy H.B., Mueller J.M., Schlitter E., Schwyzer R. and St. André A.F. - Helv. Chim. Acta 37: 59 (1954).

14. Lucas R.A., Kuehne M.E., Ceylowski M.J., Dziemian R.L. and MacPhillamy H.B. - J. Amer. Chem. Soc. 81: 1928 (1959).