dine, c 0.74) and $[\alpha]_D$ −81° (chloroform, c 0.57); O.R.D. in chloroform (c 0.205): $\{\alpha\}_{578}$ −78.2°, $[\alpha]_{546}$ −91.1°, $[\alpha]_{435}$ −190, $[\alpha]_{405}$ −284°, and $[\alpha]_{365}$ −485°[43]; ν_{max}^{Nujol} 3430 (72), 1706 (88), and 749 cm.$^{-1}$ (90%).

Anal. Calcd. for $C_{20}H_{24}N_2O$: C, 77.88; H, 7.84; N, 9.08. Found: C, 77.80; H, 7.92; N, 9.20.

Further elution of the column with chloroform followed by chloroform containing 1 and 5% methanol, respectively, yielded 1.75 g. of the starting alcohol as was shown by its paper chromatogram and infrared spectrum.

3ξ-Benzylyohimbane (IIIe).—Benzylmagnesium bromide was prepared by dropwise addition of a solution of 34 g. of benzyl bromide in 400 ml. ether to a stirred suspension of 15.8 g. of magnesium in 100 ml. of ether. When addition was completed the mixture was stirred at room temperature for 1 hr. longer and then refluxed for 45 min. To the Grignard reagent was added 12 g. (0.038 mole) of 3-dehydroyohimbane chloride (IIa). The reaction mixture was stirred at room temperature for 4 hr., allowed to stand at room temperature overnight, and was then poured into a solution of 30 g. of ammonium chloride in 600 ml. of ice water. After separation of the layers, the aqueous layer was extracted with several portions of ether, the combined ether solutions were dried over sodium sulfate, and were evaporated to dryness. The residue was dissolved in 200 ml. of 50% acetic acid, water was added to reduce the acetic acid concentration to 15%, and partial neutralization to pH 4 was carried out by the addition of ammonium hydroxide. The aqueous solution was decanted from the gum which formed and the latter was dissolved in methylene chloride. This solution was dried over sodium sulfate, evaporated to dryness, and the residue was crystallized from methanol to give 6.7 g. of product, m.p. 221–222° dec., $[\alpha]_D$ −193° (chloroform, c 0.55). In addition there was obtained 0.8 g. of a second crop, m.p. 214–216° dec., $[\alpha]_D$ −199° (chloroform, c 0.54). Recrystallization of the first crop from methanol gave material: m.p. 222–223° dec.; $[\alpha]_D$ −192° (chloroform, c 0.56) and $[\alpha]_D$ −4° (pyridine, c 0.51); pK_A' (70% ethanol) 6.65; ν_{max}^{Nujol} 3420 (56), 1600 (23), 760 (50), 740 (79), and 730 cm.$^{-1}$ (50%); λ_{max} 225 mμ (ϵ 39,000), 275 sh (7500), 282–283 (8000), and 240 (7000); λ_{min} 253 mμ (ϵ 3500) and 288 (6800).

Anal. Calcd. for $C_{26}H_{30}N_2$: C, 84.28; H, 8.16; N, 7.56. Found: C, 84.05; H, 8.29; N, 7.76.

Attempted Reaction of 3-Dehydroyohimbane Base (Va) with Methyllithium.—A mixture of 150 mg. of 3-dehydroyohimbane base, 0.025 mole of methyllithium in 20 ml. of ether, and 15 ml. of benzene was refluxed with stirring for 24 hr. The reaction mixture was poured on ice–water, the layers were separated, and the aqueous layer was extracted with ether. The combined organic layers were dried over sodium sulfate and evaporated to dryness. Ionophoresis on the gummy residue showed only the spot corresponding to 3-dehydroyohimbane with the slower spot characteristic of 3ξ-methylyohimbane absent. Ionograms after the same reaction was carried out with 3-dehydroyohimbane chloride or perchlorate always showed, quite prominently, this slower spot.

Attempted Reaction of 3-Dehydro-17β-hydroxyyohimbane (Vb) with Methyllithium.—A mixture of 500 mg. of 3-dehydro-17β-hydroxyyohimbane base, 0.1 mole of methyllithium in 75 ml. of ether, and 30 ml. of benzene was refluxed for 24 hr. The reaction mixture was poured into ice–water, the layers were separated, and the aqueous layer was extracted with several portions of ether. The combined organic layers were dried over sodium sulfate and evaporated to dryness. Ionophoresis of the residue showed only the spot corresponding to 3-dehydro-17β-hydroxyyohimbane with the slower spot characteristic of 3ξ-methyl-17β-hydroxyyohimbane absent. Ionograms after the same reaction was carried out with 3-dehydro-17β-hydroxyyohimbane chloride always showed, quite prominently, this slower spot.

17β-Hydroxy-3-epiyohimbane.—A mixture of 1.5 g. (0.05 mole) of 3-epiyohimbone,[5] 1.0 g. of potassium borohydride, and 100 ml. of methanol was stirred at room temperature for 18 hr. Most of the methanol was removed by distillation *in vacuo* and the residue was partitioned between water and methylene chloride. The methylene chloride solution was dried over sodium sulfate and concentrated to a small volume whereupon crystals separated. These were collected and recrystallized from acetonitrile to give 0.8 g. of product: m.p. 178–180° dec.; $[\alpha]_D$ −24° (pyridine, c 0.55); ν_{max}^{Nujol} 3400 (57), 3160 (69), and 738 cm.$^{-1}$ (81%).

Anal. Calcd. for $C_{19}H_{24}N_2O$: C, 76.99; H, 8.16; N, 9.45. Found: C, 76.81; H, 8.30; N, 9.21.

***t*-Butyl Hypochlorite Oxidation of Yohimbane, 3ξ-Methylyohimbane (IIIa), 3ξ-Phenylyohimbane (IIIb), and 3ξ-Benzylyohimbane (IIIe).**—Solutions of 0.0005 mole of each of these compounds in a mixture of 60 mg. (0.06 mole) of triethylamine and 15 ml. of methylene chloride were cooled to −5°. To each flask was added a solution of 75 mg. (0.069 mole) of *t*-butyl hypochlorite in 1 ml. of methylene chloride. The reaction mixtures were allowed to stand at −5 to 0° for 20 min. and the resulting solutions were washed with two 10-ml. portions of water. The organic layers were dried over sodium sulfate and evaporated *in vacuo* to give residues which were dissolved in 10 ml. of 0.8 *N* ethanolic hydrogen chloride. Appropriate dilutions of these solutions were used for the determination of the ultraviolet spectra. It was found that only the product derived from yohimbane gave a strong maximum at 350 mμ. The other products were devoid of absorption in this region.

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(43) O.R.D. of yohimbone in chloroform (c 0.209): $[\alpha]_{578}$ −94.7°, $[\alpha]_{546}$ −106°, $[\alpha]_{435}$ −201°, $[\alpha]_{405}$ −281°, and $[\alpha]_{365}$ −440°. O.R.D. of pseudoyohimbone in chloroform (c 0.209): $[\alpha]_{578}$ −34.9°, $[\alpha]_{546}$ −47.9°, $[\alpha]_{435}$ −129°, $[\alpha]_{405}$ −213°, and $[\alpha]_{365}$ −398°.

Fonsecin, a Pigment from an *Aspergillus fonsecaeus* Mutant

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Fonsecin, a yellow pigment produced by an ultraviolet mutant of the fungus *Aspergillus fonsecaeus*, is shown to be the previously unknown 2-methyl-2,5,8-trihydroxy-6-methoxy-2,3-dihydro-4H-naphtho[2,3-*b*]pyran-4-one (I).

Aspergillus fonsecaeus, a fungus closely related to *A. niger*, normally produces a brown-black pigment.[3] In 1953 Raper and Fennell[4] reported that a number of lighter colored mutants could be obtained from *A. fonsecaeus* subjected to ultraviolet radiation. One of these mutants (O 16–1) is bright yellow and grows well on various substrata. It is, however, very short lived,

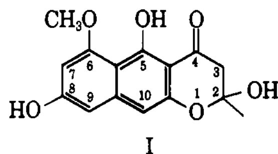
(1) To whom inquiries should be addressed.

(2) One of the laboratories of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

(3) C. Thom and K. B. Raper, "A Manual of the Aspergilli," Williams and Wilkins Co., Baltimore, Md., 1945, p. 227.

(4) K. B. Raper and D. I. Fennell, *J. Elisha Mitchell Sci. Soc.*, **69**, 1 (1953).

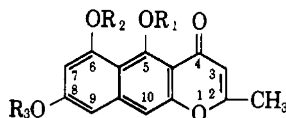
requiring retransfer at monthly intervals. Its intense pigmentation results from a heavy deposit of crystalline coloring matter in the vesicles and primary sterigmata. In this paper we describe the isolation of fonsecin, the principal pigment produced by this mutant, and present evidence that it is 2-methyl-2,5,8-trihydroxy-6-methoxy-2,3-dihydro-4H-naphtho[2,3-*b*]pyran-4-one (I). In a preliminary note[5] fonsecin was assigned a provisional formula which was recently shown by the synthetic work of Shibata, Morishita, and Arima[6] to be untenable.



A. fonsecaeus was grown for 17 days at 25° in Fernbach flasks on a medium containing sucrose and steep liquor, and the yellow mycelium was separated by filtration. The dried mat was extracted with ethyl acetate to give a crude product, which was shown by paper chromatography to consist of at least four different pigments. Recrystallization gave pure fonsecin in an amount corresponding to about 4% of the dried mycelium.

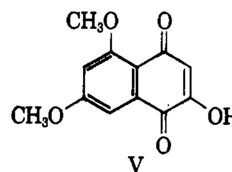
Fonsecin crystallizes as irregular, bright yellow prisms from ethyl acetate–petroleum ether and melts at 198° with decomposition. It is rather soluble (20–30 mg./ml.) in acetone, ethanol, methanol, and ethyl acetate; difficultly soluble (*ca.* 1 mg./ml.) in benzene, chloroform, and ethyl ether; and almost insoluble in petroleum ether and water. Fonsecin dissolves readily in dilute aqueous sodium hydroxide, forming a red solution, but is insoluble in a saturated sodium carbonate solution. It produces a green color with alcoholic ferric chloride and shows a positive coupling reaction with diazotized benzidine.

Formula I for fonsecin was deduced from the following data. Elementary analyses and molecular weight determinations are in agreement with a molecular formula of $C_{15}H_{14}O_6$ for fonsecin, which shows one methoxyl group and one C-methyl group. Demethylation of fonsecin yields norrubrofusarin (II), the structure of which follows from that of rubrofusarin (III) established by Stout, Dreyer, and Jensen[7] by X-ray methods and chemically by Tanaka and co-workers.[8] Complete methylation of fonsecin gives rubrofusarin dimethyl ether (IV), which is further evidence for close relationship between fonsecin and rubrofusarin.



II (norrubrofusarin), $R_1 = R_2 = R_3 = H$
III (rubrofusarin), $R_1 = R_2 = H$; $R_3 = CH_3$
IV (rubrofusarin dimethyl ether), $R_1 = R_2 = R_3 = CH_3$
VI (rubrofusarin diacetate), $R_1 = R_2 = CH_3CO$; $R_3 = CH_3$
VII (diacetate from fonsecin), $R_1 = R_3 = CH_3CO$; $R_2 = CH_3$
VIII (acetate from fonsecin monomethyl ether), $R_1 = CH_3CO$; $R_2 = R_3 = CH_3$

Mild treatment of fonsecin with diazomethane produces a monomethyl ether of m.p. 176° having the composition $C_{16}H_{16}O_6$, a reaction in which there is no loss of water, in contrast with the preparation of the dimethyl ether from fonsecin. That the two methoxyl groups in fonsecin monomethyl ether occupy the C-6 and C-8 positions follows from the fact that mild oxidation of this ether gives 5,7-di-O-methylflaviolin (V), as demonstrated by paper chromatography and isolation of tri-O-methylflaviolin. This evidence is further supported by the observation that 3,5-dimethoxyphthalic anhydride results from permanganate oxidation of the monomethyl ether.



If the methoxyl group of fonsecin were at C-8, then diacetylation of fonsecin with acetic anhydride and pyridine should yield the known[9] rubrofusarin diacetate (VI) of m.p. 260°, assuming loss of water; instead, an isomeric compound of m.p. 204–206° is obtained, which must have structure VII.

The n.m.r. spectrum of fonsecin is in agreement with structure I: τ 8.28 (singlet, three protons, C-2 methyl), 7.03 (two protons, C-3 methylene), 5.98 (three protons, C-6 methoxy), aromatic signals centered at 3.48, equivalent to three protons, and three hydroxyl signals, each equivalent to one proton (C-2 at 3.73, C-5 at −4.68, and C-8 at 0.37). The other possible structure, in which the hydroxyl group is at C-3, is eliminated by the fact that the methyl group at C-2 shows no splitting due to a *gem* proton. The hydrate structure proposed in our note[5] is ruled out because no olefinic proton signal is observed between τ 4–5.

The easy formation of acetone by alkaline hydrolysis of fonsecin and its monomethyl ether is also in accord with formula I.

The easy dehydration of fonsecin observed in this work has its parallel in the conversion of 2-hydroxychromanone compounds to the corresponding chromones reported by Narasimhachari, *et al.*[10] Besides the diacetate from fonsecin already described, the acetate from fonsecin monomethyl ether (VIII) should be mentioned as a product of such a dehydration.

Experimental

Melting points were capillary and uncorrected; ultraviolet spectra were determined on a Beckman DU spectrophotometer[11] in 95% alcohol; infrared spectra, on a Perkin-Elmer Model 21 in KBr disks; n.m.r., on a Varian Associates 60 Mc. high-resolution spectrometer in deuteriochloroform–deuteriodimethyl sulfoxide; and paper chromatography, on Whatman No. 1 paper with benzene–acetic acid–water (2:2:1), unless otherwise noted.

(5) O. L. Galmarini, F. H. Stodola, K. B. Raper, and D. I. Fennell, *Nature*, **195**, 502 (1962).

(6) S. Shibata, E. Morishita, and Y. Arima, *Chem. Pharm. Bull.* (Tokyo), **11**, 821 (1963).

(7) G. H. Stout, D. L. Dreyer, and L. H. Jensen, *Chem. Ind.* (London), 289 (1961); *Acta Cryst.*, **15**, 451 (1962).

(8) H. Tanaka and T. Tamura, *Tetrahedron Letters*, **No. 4**, 151 (1961); *Agr. Biol. Chem.* (Tokyo), **26**, 767 (1962); H. Tanaka, Y. Ohne, N. Ogawa, and T. Tamura, *ibid.*, **27**, 48 (1963); H. Tanaka and T. Tamura, *ibid.*, **27**, 249 (1963).

(9) J. N. Ashley, B. C. Hobbs, and H. Raistrick, *Biochem. J.*, **31**, 385 (1937).

(10) N. Narasimhachari, D. Rajagopalan, and T. R. Seshadri, *J. Sci. Ind. Res.* (India), **11B**, 347 (1952); *ibid.*, **12**, 287 (1953).

(11) The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

Isolation of Fonsecin.—The fermentation medium was prepared by the addition of the following to 1 l. of water: 30 g. of sucrose, 3 g. of $NaNO_3$, 1 g. of K_2HPO_4, 0.5 g. of $MgSO_4$, 0.5 g. of KCl, 0.01 g. of $FeSO_4$, and 10 ml. of steep liquor. Each 2-l. Fernbach flask, containing 500 ml. of this medium, was inoculated with mycelium of the O 16-1 strain grown for 3 days on an agar medium containing the nutrients given above. The fermentation was continued for 17 days at 25°.

The mycelium from 15 flasks was obtained by filtration, air-dried (74.1 g.), ground to a powder, and extracted twice at room temperature for 4 hr. with 350-ml. portions of ethyl acetate. Concentration gave 18.1 g. (24.4%) of yellow powder. A portion (4 g.) was dissolved in 100 ml. of boiling ethyl acetate; the solution was treated with carbon and filtered. To the filtrate at room temperature was added slowly 200 ml. of petroleum ether (b.p. 60–70°) with mechanical stirring. After 15 min. more stirring, the clear solution was decanted from the gummy precipitate and treated as previously with 400 ml. of petroleum ether. After 2 hr. of stirring, the solution, which contained a yellow powder, was refrigerated overnight. Filtration gave crude fonsecin of m.p. 150–175°, which on crystallization from ethyl acetate–petroleum ether yielded pure fonsecin as irregular yellow prisms of m.p. 198° dec. The remainder of the 18.1 g. was purified in the same way to give a total of 3.56 g. (4.8% of mycelium weight) of pure material; λ_{max} 233 mμ (ε 26,400), 275 (31,400), 321 (7300), 332 (8030), and 400 (8780).

Anal. Calcd. for $C_{15}H_{14}O_6$: C, 62.07; H, 4.86; 1 OCH_3, 10.7; 1 C–Me, 5.2; mol. wt., 290. Found: C, 62.3, 62.1; H, 4.94, 4.79; OCH_3, 11.4; C–Me, 6.2; mol. wt. (Signer), 282, 303.

There was no weight loss on drying for 2 hr. at 110° (1 mm., P_2O_5). The gummy precipitate mentioned above represented about 8% of the dried mycelium. Paper chromatography showed that it contained at least three other yellow pigments (R_f 0.51, 0.81, and 0.91) besides fonsecin (R_f 0.30) (see Table I).

TABLE I

PAPER CHROMATOGRAPHY OF FONSECIN AND RUBROFUSARIN COMPOUNDS

Compounds	R_f	Ultraviolet fluorescence	Diazotized benzodine spray
Rubrofusarin	0.92	Red-brown	Brown
Fonsecin	0.30	Intense yellow	Red-brown
Rubrofusarin monomethyl ether	0.90	Light yellow	Violet
Fonsecin monomethyl ether	0.82	Light yellow	Red-brown
Rubrofusarin dimethyl ether	0.88	Light yellow	Negative

Acetone from Fonsecin.—Fonsecin (50 mg.) was dissolved in 10 ml. of 10% sodium hydroxide and refluxed. During the refluxing, nitrogen was bubbled through the reaction mixture and into a solution of 2,4-dinitrophenylhydrazine. The precipitate that formed was collected and identified as the 2,4-dinitrophenylhydrazone of acetone (melting point and mixture melting point).

Norrubrofusarin (II) from Fonsecin.—Fonsecin (700 mg.) was heated in a stream of nitrogen at 95° for 2.5 hr. with hydriodic acid (35 ml., *d.* 1.7); the solution was cooled and then poured into 350 ml. of water. The crude product (582 mg.) crystallized from ethanol as hexagonal orange-red plates, m.p. 297°, with darkening at 250–260°; λ_{max} 224 mμ (ε 30,200), 270 (46,000), 325 (3570), 340 (4200), and 273 (6030), with an inflection at 249 (ε 24,100).

Anal. Calcd. for $C_{14}H_{10}O_5$: C, 65.12; H, 3.87. Found: C, 64.7; H, 4.20.

This demethylated product was shown by mixture melting points, ultraviolet, and infrared spectra to be identical with norrubrofusarin prepared from rubrofusarin according to the method of Ashley, *et al.*[9]

The norrubrofusarin from fonsecin was converted to a dimethyl ether which had the same melting point (204–205°), ultraviolet spectrum [λ_{max} 225 mμ (ε 29,600), 274 (56,500), 330 (3280), 345 (3430), and 385 (5350)], and infrared spectrum as rubrofusarin monomethyl ether prepared by the method of Ashley, *et al.*[9]

Rubrofusarin Dimethyl Ether (IV) from Fonsecin.—Fonsecin (300 mg.) suspended in ethanol (4 ml.) was treated with excess ethereal diazomethane and kept at room temperature for 2 days. After removal of the solvent, the diazomethane treatment was repeated on the residue. The product (329 mg.) gave no coupling reaction with diazotized benzidine and only a single yellow fluorescent spot (R_f 0.90) on paper. Chromatography on grade II acid-washed alumina (eluent benzene–methanol, 9:1) yielded 128 mg. of crude dimethyl ether. Carbon treatment and crystallization from 50% methanol gave 21 mg. of colorless prisms, m.p. 186–187°. The compound was also prepared from fonsecin monomethyl ether by the same procedure. The ultraviolet and infrared absorption spectra of fonsecin dimethyl ether were identical with those published by Dreyer[12] for rubrofusarin dimethyl ether.

Anal. Calcd. for $C_{17}H_{16}O_5$: C, 67.99; H, 5.37; 3 OCH_3, 31.0. Found: C, 68.3; H, 5.71; OCH_3, 31.2.

The compound showed negative tests with ferric chloride and diazotized benzidine, turned violet with alkaline *m*-dinitrobenzene,[8] red-violet with concentrated potassium hydroxide,[13] and gave a styryl derivative with piperonal[8]; m.p. 254°.

Fonsecin Monomethyl Ether.—Fonsecin (200 mg.) dissolved in ether–ethanol (3:1) was treated with an excess of diazomethane, and the reaction mixture was refrigerated overnight. Removal of solvent left a yellow-brown residue (233 mg.), which was crystallized from benzene after carbon treatment and, finally, from methanol. The yellow prisms melted at 176°, gave a green color with alcoholic ferric chloride, and a red color with diazotized benzidine; R_f 0.77 (yellow-orange fluorescence); λ_{max} 232 mμ (ε 28,500), 277 (40,500), 317 (9100), 330 (10,000), and 395 (8400). There was no weight loss on drying at 110° for 2 hr. (1 mm., P_2O_5).

Anal. Calcd. for $C_{16}H_{16}O_6$: C, 63.16; H, 5.26; 2 OCH_3, 20.4; 1 C–Me, 4.94. Found: C, 63.1, 63.3; H, 5.28, 5.37; OCH_3, 20.6; C–Me 4.7.

Acetate from Fonsecin Monomethyl Ether (VIII).—Fonsecin monomethyl ether (200 mg.) was dissolved in acetic anhydride (2 ml.) and pyridine (8 ml.) and left overnight at room temperature. The crude acetate (201 mg.) was chromatographed on grade II acid-washed alumina (chloroform eluent). The yellow band (161 mg., m.p. 198–200°) was rechromatographed as before, and the product was crystallized twice from ethanol to yield pale yellow needles, m.p. 198–200°; λ_{max} 225 mμ (ε 22,400), 268 (35,800), and 355 (6300), with an inflection at 247 (30,300); negative $FeCl_3$ and coupling reactions.

Anal. Calcd. for $C_{18}H_{16}O_6$: C, 65.85; H, 4.87; 1 CH_3CO, 13.1; 2 OCH_3, 18.9. Found: C, 65.3; H, 4.90; CH_3CO, 11.2; OCH_3, 16.8.

Mild Oxidation of Fonsecin Monomethyl Ether.—The following procedure was kindly supplied by Dr. J. C. Roberts in advance of publication.[14] Fonsecin monomethyl ether (50 mg.) was dissolved in 11 ml. of a 23% aqueous solution of tetraethylammonium hydroxide, and the solution was added slowly to a refluxing aqueous solution of 3% potassium hydroxide (20 ml.). At the same time nitrogen was passed through the reaction mixture into a 2,4-dinitrophenylhydrazine solution. The precipitate that formed was collected, recrystallized from ethanol, and sublimed twice (100°, 0.07 mm.); m.p. 124–125°, mixture melting point with acetone 2,4-dinitrophenylhydrazone: 124–125°. Refluxing was continued for a total of 30 min.

The alkaline solution was exposed to air at room temperature overnight and then extracted with ether. The ethereal solution was extracted with saturated aqueous sodium bicarbonate; the bicarbonate extracts were acidified and re-extracted with ether. The amorphous yellow powder (18 mg.) obtained on removal of ether showed the same behavior on paper as an authentic sample of 5,7-O-dimethylflaviolin [three solvent systems: ethyl acetate–acetic acid–water (2:2:1), R_f 0.84; benzene–acetic acid–water (2:2:1), R_f 0.63; and 1-butanol–morpholine–water (100:10:15), R_f 0.39; spray: 2% sodium hydroxide solution or diazotized sulfanilic acid).

The remainder of the amorphous yellow material was refluxed with 5 ml. of 3% hydrochloric acid in methanol, the solution was evaporated to dryness, and the residue was dissolved in benzene (5 ml.) and chromatographed on acid-washed alumina (grade IV). The material from the yellow band was sublimed (155–160°, 0.07 mm.) and washed with petroleum ether and ethanol.

(12) D. L. Dreyer, University Microfilms, L. C. Card No. Mic 60-4282; *Dissertation Abstr.*, **21**, 1373 (1960); *Chem. Abstr.*, **55**, 6475 (1961).

(13) A. Schönberg and A. Sina, *J. Am. Chem. Soc.*, **72**, 1611 (1950).

(14) B. W. Bycroft, T. A. Dodson, and J. C. Roberts, *J. Chem. Soc.* 40 (1962).

Yellow needles (4 mg.) of melting point 191–192° (Kofler) were obtained; ultraviolet absorption: λ_{max}^{EtOH} 216 mμ (ε 30,800), 263 (15,700), 298 (11,200), and 415 (2800); lit.[14] (for the tri-O-methylflaviolin) m.p. 191–192°; λ_{max}^{EtOH} 215 mμ (ε 31,600), 262 (15,100), 298 (11,000), and 414 (2690).

Permanganate Oxidation of Fonsecin Monomethyl Ether.—Fonsecin monomethyl ether (20 mg.) in 0.3 *N* sodium hydroxide (7.5 ml.) was oxidized with 5% $KMnO_4$ solution (1.2 ml.) according to the method of Ebnöther, *et al.*[15]

The crude acid fraction showed a light blue acidic spot (0.03% methyl red in 0.05 *N* borate buffer) with R_f 0.32, the same as for synthetic 3,5-dimethoxyphthalic acid. The rate of migration with methyl ethyl ketone–water–pyridine (92.1:7.7:0.2) was also the same as for the synthetic acid, 0.82. The oxidation of rubrofusarin monomethyl ether yielded no detectable 3,5-dimethoxyphthalic acid.

3,5-Dimethoxyphthalic anhydride was isolated by the following procedure. Fonsecin monomethyl ether (40 mg.) was hydrolyzed as described above in the preceding section. The ether-extractable material (25 mg.) was dissolved in 2 *N* sodium hydroxide (4 ml.), 15% hydrogen peroxide (2.2 ml.) was added, and the reaction mixture was kept 1 hr. at room temperature before being heated on a steam bath for 30 min. After the solution was acidified and extracted with ether, the ethereal residue was heated at 100° for 30 min. with acetic anhydride (2 ml.). Evaporation to dryness and three sublimations (110°, 0.07 mm.) gave colorless needles (3 mg.), m.p. 148–149°, which showed no depression in melting point on admixture with an authentic sample of 3,5-dimethoxyphthalic anhydride of the same melting point.

(15) A. Ebnöther, T. H. Maijer, and H. Schmid, *Helv. Chim. Acta*, **35**, 910 (1952).

Diacetate from Fonsecin (VII). A.—Fonsecin (300 mg.) was refluxed for 2 hr. with acetic anhydride (30 ml.) and anhydrous sodium acetate (3 g.). The crude acetate (280 mg.) was chromatographed on grade II acid-washed alumina (chloroform eluent). The yellow band (172 mg.) was rechromatographed in the same way to yield 136 mg. of product which on crystallization from alcohol-benzene gave colorless needles of diacetyl fonsecin: m.p. 204–206°; negative $FeCl_3$ test; λ_{max} 260 mμ (ε 40,400), 315 (3960), and 358 (6530), with an inflection at 240 (27,000).

Anal. Calcd. for $C_{19}H_{16}O_7$: C, 64.04; H, 4.49; 2 CH_3CO, 24.2. Found: C, 63.6; H, 4.40; CH_3CO, 20.0.

B.—Fonsecin (500 mg.) was heated at 100° for 5 hr. with acetic anhydride (5 ml.) and pyridine (0.07 ml.). The crude acetate (585 mg.) was chromatographed on Magnesol (acetone eluent). The yellow band (170 mg., m.p. 204°) was rechromatographed in the same way to give 144 mg. of product which on crystallization from ethanol–benzene gave colorless needles of m.p. 206–208°, showing a correct analysis for $C_{19}H_{16}O_7$.

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Structure and Synthesis of Lathyrine[1]

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The structure of lathyrine (I) has been confirmed to be β-(2-aminopyrimidin-4-yl)alanine by additional chemical studies and by synthesis. Lathyrine has been converted to the hydrochloride, monobenzoyl, and tetrahydro derivatives. Alkaline permanganate oxidation of I yielded 2-amino-4-carboxypyrimidine (III), identical with III obtained by oxidizing 2-amino-4-methylpyrimidine (II). Condensation of 2-diacetylamino-4-methylpyrimidine with ethyl oxalate, followed by treatment of the product with hydroxylamine gave oximino ester VI. Hydrolysis of VI to the corresponding (oximino) acid followed by reduction with stannous chloride gave *dl*-I.

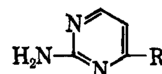
β-(2-Aminopyrimidin-4-yl)alanine has been proposed by Bell and Foster[2] as the structure of lathyrine (I), an amino acid isolated[2,3] from seeds of *Lathyrus tingitanus* (Tangier pea) and distinguished by its unusual red color with ninhydrin. Although the proposed structure was based on firm physical evidence, *e.g.*, close ultraviolet similarity to 2-amino-4-methylpyrimidine (II), and n.m.r. spectral data, the only analyzed chemical derivatives were a sulfate salt and a hydrogenation product, the latter derived from I by uptake of 2 moles of hydrogen. The purpose of the present study was to explore further the chemistry of lathyrine and to undertake its synthesis. We also prepared a hydrochloride salt and a monobenzoyl derivative of I as well as a crystalline hydrochloride salt of reduced lathyrine.

(1) Supported in part by Grant G-22249 from the National Science Foundation. Presented in part at the 145th National Meeting of the American Chemical Society, New York, N. Y., Sept. 1963.

(2) E. A. Bell and R. G. Foster, *Nature*, **194**, 91 (1962); E. A. Bell, *Biochim. Biophys. Acta*, **47**, 602 (1961).

(3) E. Nowacki and J. Przybylska, *Bull. Acad. Polon. Sci. Ser. Sci. Biol.*, **9**, 279 (1961). These authors, who independently isolated the amino acid, called it "tingitanine."

By oxidation of I with alkaline permanganate to the known pyrimidine, 2-amino-4-carboxypyrimidine (III),[4] the pattern of ring substitution was chemically confirmed. Finally the complete structure was verified by synthesis of *dl*-I.



I, R = $CH_2CH(NH_2)COOH$
II, R = CH_3
III, R = COOH

The route chosen for synthesis of lathyrine is outlined in Chart I.[5]

Acetylation of 2-amino-4-methylpyrimidine (II) with acetic anhydride gave 2-diacetylamino-4-methylpyrimidine (IV) as the major product, and 2-acetyl-

(4) T. Matsukawa and K. Shirakawa, *J. Pharm. Soc. Japan*, **72**, 909 (1952).

(5) An attempt to prepare 2-diacetylamino-4-bromomethylpyrimidine for reaction with acetamidomalonate was unsuccessful. Reaction of IV with N-bromosuccinimide gave a low yield of 2-diacetylamino-4-dibromomethylpyrimidine, m.p. 109–111° (*Anal.* Calcd. for $C_9H_9Br_2N_3O_2$: N, 11.97; Br, 45.53. Found: N, 12.04; Br, 46.01).