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 $C_{19}H_{16}O_7$.

Acknowledgment.—The authors thank Drs. H. Raistrick and J. H. Birkinshaw of the London School of Hygiene and Tropical Medicine, Professor G. H. Stout of the University of Washington, and Dr. J. C. Roberts of the University of Nottingham for reference compounds; Dr. E. H. Melvin and Curtis Glass for spectral determinations; Dr. C. W. Hesseltine for fermentation work; Dr. R. G. Pitcher of Varian Associates for the n.m.r. spectrum and its interpretation; and Mrs. C. E. McGrew for microanalyses. The work of one of us (O. L. G.) was generously supported by the John Simon Guggenheim Memorial foundation.

Structure and Synthesis of Lathyrine[1]

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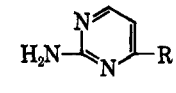
The structure of lathyrine (I) has been confirmed to be β-(2-aminopyrimidin-4-yl)alanine by additional chemical studies and by synthesis. Lathyrine has been converted to the hydrochloride, monobenzoyl, and tetrahydro derivatives. Alkaline permanganate oxidation of I yielded 2-amino-4-carboxypyrimidine (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.

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-

(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.

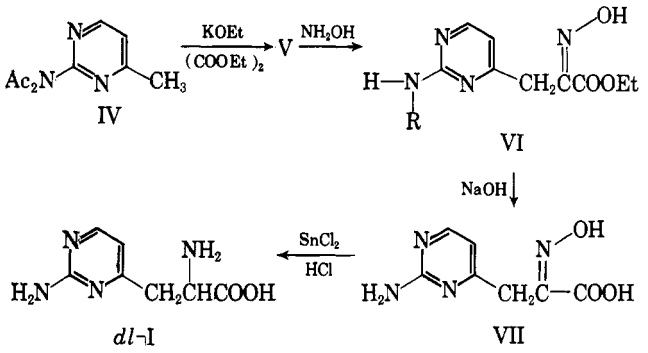
(2) E. A. Bell and R. G. Foster, *Nature*, **194**, 91 (1962); E. A. Bell, *Biochim. Biophys. Acta*, **47**, 602 (1961).

(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."

(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).

CHART I



amino-4-methylpyrimidine. These compounds, which were separated by fractional crystallization, were identified by infrared and n.m.r. spectra, and by microanalyses.

Claisen condensation of 2-diacetylamino-4-methylpyrimidine (IV) with ethyl oxalate afforded a yellow solid (V) possessing a characteristic visible spectrum with maxima at 422, 403, and 330 mμ in ethanol. Product V, which was not homogeneous by thin layer chromatography, was assumed to be a mixture of acetylated derivatives of ethyl 2-aminopyrimidin-4-ylpyruvate, or one of its tautomers.[6]

By reaction with hydroxylamine, V was converted to the oximino ester VI. Thin layer chromatography indicated that this product also was a mixture. Fractional crystallization of the reaction products derived from various batches of V eventually led to two homogeneous substances, VIa, m.p. 148–149°, and VIb, m.p. 182–185°. The former possessed characteristic ultraviolet[7] (absorptions at 226 and 295 mμ shifting to 220 and 301 mμ upon acidification), infrared[8] [absorptions at 3.0 and 3.1 μ (N–H stretch)], and n.m.r. (see Experimental) spectra to be expected for the oxime of ethyl 2-aminopyrimidin-4-ylpyruvate (VI, R = H). Comparison of ultraviolet [absorptions at 232 and 264 (shoulder) mμ, shifting to 232 and 278 mμ in acid] and infrared (absorptions at 3.16 and 3.36 μ) spectra of VIb, with that of 2-acetylamino-4-methylpyrimidine suggested that VIb is the oxime of ethyl 2-acetylaminopyrimidine-4-ylpyruvate (VI, R = Ac).[9]

Saponification of VI to the oximino acid VII followed by reduction of VII with stannous chloride gave *dl*-β-(2-aminopyrimidin-4-yl)alanine (I) as the hydrochloride salt. This salt was indistinguishable from the hydrochloride of natural lathyrine in ultraviolet and infrared spectra and chromatographic behavior in several solvent systems. A single symmetrical peak, with D_{440}/D_{570} ratio of 2.5, was observed when individual samples of synthetic and natural lathyrine, and a mixture of the two were chromatographed in an amino acid analyzer.

(6) The visible spectrum of V suggests that this product exists in a tautomeric form of the α-keto ester. Similar conclusions have been drawn by E. C. Taylor and E. S. Hand, [*J. Am. Chem. Soc.*, **85**, 770 (1963)] for ethyl 3-methylquinoxal-2-ylpyruvate.

(7) S. F. Mason in "The Pyrimidines," D. J. Brown, Ed., Interscience Publishers, Inc., New York, N. Y., 1962, pp. 492

(8) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd Ed., Methuen and Co., Ltd., London, 1958, pp. 5–9.

(9) Both VIa and VIb were separately converted to the oximino acid VII (identical infrared spectra) by treatment with 1 *N* sodium hydroxide.

Experimental[10]

Isolation of Lathyrine.—The isolation procedure of Nowacki[3] was used since better yields were obtained than by the procedure of Bell.[2] Batches of *Lathyrus tingitanus* seeds[11] (0.5–1.5 kg.), which had been ground in a large Wiley mill, were extracted seven times with methanol at 25° using 1 ml. of methanol per gram of seeds each time. The combined methanol extracts were evaporated *in vacuo* to a small volume (50–150 ml.), diluted with an equal volume of water, and shaken thoroughly with 10 vol. of ether. Upon standing in the icebox (4°), crystals of crude amino acid (yield 4.7–7.3 g./kg. of seeds) gradually formed in the aqueous layer. After recrystallization from water, the lathyrine obtained was identical with samples isolated by Bell and Nowacki[12] in spectral properties and chromatographic behavior. The melting point of lathyrine has been reported to be 215° dec.[2] and 296° dec.[3] In the present study when the crystals were observed on a hot stage melting point apparatus, gradual darkening between 260–300° occurred with no evident liquefaction or gas evolution. Gradual darkening was observed in a capillary tube above 240° with no definite melting or decomposition point.

A highly purified lathyrine sample was readily obtained by adsorption on Dowex-1 (acetate form) resin, elution by sodium acetate, and direct crystallization from the concentrated eluate.

The hydrochloride salt was prepared (for comparison with the synthetic product) by dissolving natural lathyrine in 2 equiv. of 2 *N* hydrochloric acid, and inducing crystallization by addition of acetone. The recrystallized (acetone–water) monohydrochloride had $[\alpha]^{27}D$ −14.4° (*c* 1.7, water); $\lambda_{max}^{pH\ 1}$ 298 mμ (ε 4370) and 222 mμ (ε 15,100); $\lambda_{max}^{pH\ 13}$ 292 mμ (ε 4370) and 226 mμ (ε 13,000); λ_{max}^{KBr} 2.9, 3.07, 3.27, 2.9–4.5 (broad), 5.79, 6.03, and 6.20 μ.

Anal. Calcd. for $C_7H_{11}ClN_4O_2 \cdot 0.5H_2O$: C, 36.93; H, 5.31; Cl, 15.57; N, 24.62. Found: C, 37.15; H, 5.36; Cl, 15.68; N, 24.91.

Preparation of Benzoyl Derivative of Lathyrine.—To a suspension of lathyrine (250 mg., 1.39 mmoles) and benzoyl chloride (0.4 ml., 3.4 mmoles) in 2 ml. of water, 4 *N* sodium hydroxide (1.4 ml.) was added dropwise with vigorous agitation. Most of the material dissolved to give a light brown solution. Upon acidifying to congo red with dilute hydrochloric acid, a gummy white precipitate separated. This was collected by centrifuging, washed several times with water, and stirred repeatedly with fresh portions of dry ether. The dried solid was recrystallized three times from hot ethanol (75 ml.) with an intermediate decolorization with carbon (yield, 148 mg.). The colorless crystalline product, m.p. 229° dec., had $\lambda_{max}^{pH\ 12}$ 293 mμ, λ_{max}^{KBr} 3.03 and 6.1 μ.

Anal. Calcd. for $C_{14}H_{14}N_4O_3$: C, 58.73; H, 4.93; N, 19.57. Found: C, 58.80; H, 5.28; N, 19.2.

Attachment of the benzoyl group to the α-amino group is presumed from failure of the derivative to give a positive reaction with ninhydrin. The benzoyl derivative with m.p. 112° reported[3] previously was not analyzed. If formed in our reaction it might conceivably have been removed in the ether washings.

When I was dissolved in acetic anhydride by warming, an intense violet color developed within a few minutes and a dark purple solid could be precipitated by addition of ether. Similar results were obtained when glacial acetic acid or pyridine were used as solvents. A sensitive color test for microgram quantities

(10) All melting points are corrected. Ultraviolet absorption spectra were observed with a Beckman DB recording spectrophotometer. Infrared spectra were observed with a Beckman IR-5 recording spectrophotometer. N.m.r. spectra (calibrated against hexamethyldisiloxane or tetramethylsilane) were recorded with a Varian Associates A-60 recording spectrometer. The authors wish to thank the University of Wisconsin Chemistry Department for the use of this spectrometer and Professor Paul Bender for assistance in obtaining the n.m.r. spectra. Optical rotations were measured with a Rudolph spectropolarimeter through the courtesy of Professor Harlan Goering.

(11) Kindly supplied by H. A. Schoth, Bureau of Plant Industry, U. S. Department of Agriculture, Corvallis, Ore.

(12) We are grateful to Professor Bell and Professor Nowacki for supplying samples of lathyrine. For the sample recrystallized from water we observed infrared maxima (KBr) at 2.96, 3.02, 3.13, 3.3–3.5, 6.09, 6.21, 6.28, 6.40, and 6.80 μ. For a sample recrystallized from glacial acetic acid bands were observed at 2.97, 2.9–4.4 (broad), 4.66, 5.9, and 6.05–6.6 (series of bands) μ. L. K. Ramachandran and K. K. Rao [*Biochim. Biophys. Acta*, **86**, 264 (1964)] have reported that 0.5 mole of acetic acid is retained by lathyrine even after overnight drying at 1 mm. and 80°.

of I thus could be observed by short-time boiling in acetic anhydride.

Hydrogenation of Lathyrine.[13]—A solution of lathyrine (1.0 g., 5.5 mmoles) in methanol (95 ml.) and 1 *N* hydrochloric acid (5.5 ml.) was hydrogenated (40 lb. pressure, Parr hydrogenation apparatus) over platinum oxide catalyst (100 mg.) until no further uptake of hydrogen was observed (8 hr.). By the end of the hydrogenation, the ultraviolet absorption at 298 mμ had disappeared and crystallization of white solid was noted. The catalyst was removed by centrifuging and the product–catalyst precipitate was washed with water to redissolve the product. The combined clear aqueous and alcohol supernatants were concentrated *in vacuo* to approximately 10 ml. Addition of methanol to persistent cloudiness of the solution followed by cooling to 0° afforded two crops (680 mg. and 200 mg.) of colorless crystals. Recrystallization from methanol gave β-(2-amino-3,4,5,6-tetrahydropyrimidin-4-yl)alanine hydrochloride,[14] m.p. 252–254°, λ_{max}^{KBr} 3.0–3.5, 5.93, and 6.13 μ.

Anal. Calcd. for $C_7H_{16}ClN_4O_2$: C, 37.75; H, 6.79; Cl, 15.92; N, 25.16; O, 14.36. Found: C, 37.71; H, 6.83; Cl, 16.18; N, 25.29; O, 14.51.

Although optical isomers might be expected, we did not determine whether a mixture of isomers was obtained.

A normal lavender color was given by tetrahydrolathyrine with ninhydrin, in contrast to the red color observed with I. Electrometric titration of tetrahydrolathyrine (kindly carried out by Ulrike Lichti) showed no pK_a' at 3.8 as was observed in I and ascribed[2] to the 2-amino group. The strongly basic character of the reduction product was indicated by its emergence between arginine and homoarginine from the Beckman–Spinco amino acid analyzer. The nonidentity of the reduced product with homoarginine was further demonstrated by thin layer chromatography on silica gel G in a 4:1 phenol–0.15 *N* ammonium hydroxide system, in which it had R_f 0.30 *vs.* 0.22 for homoarginine and 0.17 for arginine.

Tetrahydrolathyrine gave an orange color with a modified[15] Sakaguchi reagent and a purple-red with "pentacyanoaquoferriate,"[16] whereas I was completely negative to both of these tests. Unlike arginine or homoarginine it gave a negative test with diacetyl[16] reagent for monoalkyl guanidines.

Oxidation of Lathyrine.—A mixture of 364 mg. (2 mmoles) of lathyrine, 1.8 g. of potassium permanganate, and 1.8 g. of potassium hydroxide in 75 ml. of water was heated at 75° for 1.5 hr. Ethanol was added dropwise to destroy excess permanganate, and the precipitated manganese dioxide was separated by centrifuging. The aqueous solution, adjusted to pH 5 with hydrochloric acid was evaporated to dryness and the residue was dissolved in 15 ml. of water. By adding 5 vol. of ethanol a precipitate of potassium chloride was obtained. Addition of 3 vol. more of ethanol gave (upon cooling in ice) 200 mg. of needle crystals of the potassium salt of 2-amino-4-carboxypyrimidine.[17]

The potassium salt derived from lathyrine was converted to the free acid by acidification of a concentrated aqueous solution with hydrochloric acid to pH 2. The precipitated solid, after recrystallization from water (*ca.* 1.5 mg. dissolved in 1 ml. of boiling water), gave as fine needles 2-amino-4-carboxypyrimidine (III), m.p. 265° dec. (lit.[4] m.p. 285° dec.); $\lambda_{max}^{pH\ 1}$ 322 mμ (ε 4050) and 224 mμ (ε 14,200); $\lambda_{max}^{pH\ 13}$ 307 and 229 mμ; λ_{max}^{KBr} 3.08, 3.3, 5.95, and 6.15 μ. The infrared and ultraviolet spectra of this product were identical with those observed for an authentic sample of 2-amino-4-carboxypyrimidine[18] prepared from 2-amino-4-styrylpyrimidine.

Anal. Calcd. for $C_5H_5N_3O_2$: C, 43.18; H, 3.63; N, 30.21. Found: C, 42.93; H, 3.49; N, 30.11.

Oxidation of I by heating on a steam bath with concentrated nitric acid gave a crude product spectrally similar to III.

Acetylation of 2-Amino-4-methylpyrimidine (II).—A solution of 4.2 g. (0.038 mole) of II (Mann Research Laboratories, Inc.) in 125 ml. of acetic anhydride was heated under reflux for 2 hr. The solvent was removed by distillation under reduced pressure. Recrystallization of the yellow residue from benzene–petroleum ether (b.p. 60–68°) afforded 0.9 g. (first crop), m.p. 147–150°, R_f 0.7 by thin layer chromatography (t.l.c.)[19]; 3.5 g. (second crop), m.p. 95–104°; 1.2 g. (third crop), m.p. 93–128°; and 1.1-g. residue. The second and third fractions displayed two components, R_f 0.7 and 0.8 (major).

Recrystallization of the first-crop material from ethanol afforded 0.6 g. of 2-acetylamino-4-methylpyrimidine, m.p. 152–154°; $\lambda_{max}^{H_2O}$ 233 mμ (ε 15,000); $\lambda_{max}^{pH\ 1}$ 275 mμ (ε 5000) and 231 mμ (ε 17,000); λ_{max}^{KBr} 3.17, 3.33, 5.97, and 6.25 μ; n.m.r. absorptions (measured in deuterium oxide) at τ 7.87, 7.65, 5.40 (HOD), 3.00 (doublet, J = 5 c.p.s.), and 1.68 (doublet, J = 5 c.p.s.) of relative area (excluding the HOD peak) 3:3:1:1.

Anal. Calcd. for $C_7H_9N_3O$: C, 55.61; H, 6.00; N, 27.80. Found: C, 55.88; H, 5.87; N, 27.75.

Two recrystallizations of the 3.5 g. of second-crop material from benzene–petroleum ether (60–68°) afforded 2.8 g. of IV, colorless crystals, m.p. 101.5–103.5°; $\lambda_{max}^{H_2O}$ 246 mμ (ε 3500); $\lambda_{max}^{pH\ 1}$ 246 mμ (ε 3500); λ_{max}^{KBr} 5.80–5.86 and 6.30 μ; n.m.r. absorptions (measured in deuterium oxide) at τ 7.77, 7.45, 5.39 (HOD), 2.50 (doublet, J = 5–6 c.p.s.), and 1.28 (doublet, J = 5–6 c.p.s.) of relative area (excluding the HOD peak) 6:3:1:1.

Anal. Calcd. for $C_9H_{11}N_3O_2$: C, 55.94; H, 5.74; N, 21.75; O, 16.56. Found: C, 55.98; H, 6.13; N, 21.72; O, 16.47.

Oximino Ester VI.—The Claisen condensation of IV with ethyl oxalate was accomplished by a procedure similar to that described[20,21] by Pfleiderer. To a solution containing 0.37 g. (0.010 g.-atom) of potassium in 1.5 ml. of absolute ethanol and 20 ml. of anhydrous ether (nitrogen atmosphere) was added 1.3 ml. of ethyl oxalate and 0.76 g. (4 mmoles) of IV. The mixture was stirred for 4 days. The yellow paste obtained was filtered, washed with ether, and added to 0.6 ml. of glacial acetic acid in 11 ml. of water. From this mixture 0.7 g. of α-keto ester V was isolated as a yellow solid. Thin layer chromatography indicated that the product was not homogeneous. The following spectrum was observed: $\lambda_{max}^{95\%\ EtOH}$ 422, 403, 330, 286, and 230 mμ.

The procedure described by Elliott, *et al.*,[22] was followed for the conversion of α-keto ester V to α-oximino ester VI. For example, 0.7 g. of V was added to a solution containing 0.47 g. (6.8 mmoles) of hydroxylamine hydrochloride, 0.66 g. (8.0 mmoles) of sodium acetate, 4.3 ml. of water, and 14 ml. of ethanol. The mixture

(13) Catalytic hydrogenation of 2-amino-4-methylpyrimidine (II) in a similar manner yielded 2-amino-4-methyl-3,4,5,6-tetrahydropyrimidine hydrochloride (recrystallized from ethanol–ether),[14] m.p. 148–150.5° (*Anal.* Calcd. for $C_5H_{12}ClN_3$: Cl, 23.70; N, 28.08. Found: Cl, 24.40; N, 27.86.) This compound gave no color with Sakaguchi reagent,[15] but reacted similarly to tetrahydrolathyrine toward pentacyanoaquoferriate reagent.[16]

(14) H. Aft and B. E. Christensen [*J. Org. Chem.*, **27**, 2170 (1962)] determined that catalytic hydrogenation of 2-aminopyrimidines gave the corresponding 3,4,5,6-tetrahydro derivatives.

(15) J. P. Jepson and I. Smith, *Nature*, **172**, 1100 (1953).

(16) Ivor Smith, "Chromatographic and Electrophoretic Techniques," Vol. I, Interscience Publishers, Inc., New York, N. Y., 1960, Chapter 11.

(17) Oxidation of II in a similar manner afforded a potassium salt of 2-amino-4-carboxypyrimidine, identical with that obtained from lathyrine by comparison of ultraviolet, infrared (λ_{max}^{KBr} 2.87, 3.03, 3.14, 6.1–6.2, 12.20 12.65, and 14.45 μ), and n.m.r. spectra. The n.m.r. spectra of the potassium salts (measured in deuterium oxide) possessed absorptions at τ 5.32 (HOD), 2.95 (doublet, J = 5 c.p.s.), and 1.62 (doublet, J = 5 c.p.s.). Microanalyses did not enable us to assign a structure to the potassium salts. Although C–N ratios were 1.67 (theory, 1.67), the analytical values (other than potassium) were too low. This direct oxidation of II resulted in almost a quantitative yield of III, based on ultraviolet density measurements. Our attempts to prepare 2-amino-4-styrylpyrimidine[4] were unsuccessful, the only product obtained being 2-benzylamino-4-styrylpyrimidine. Long needles of the potassium salt of 2-amino-4-carboxy-5-chloropyrimidine were obtained from a commercial sample of the crude acid by dissolving in dilute potassium hydroxide, neutralizing to pH 5, removing potassium chloride with 5 vol. of alcohol, and separating the product by further addition of 3 vol. of alcohol. This crystalline salt had $\lambda_{max}^{pH\ 1}$ 323 and 233 mμ; $\lambda_{max}^{pH\ 13}$ 310 and 236 mμ; λ_{max}^{KBr} 3.01, 3.15 (N-H stretching), 12.22, 12.73, and 14.48 μ.

(18) A reference sample of this compound was kindly donated by Dr. K. Shirakawa.

(19) Unless stated otherwise, all thin layer chromatograms were obtained on silica gel G using 1:4 methanol–chloroform with iodine vapor as indicator.

(20) W. Pfleiderer and H. Mosthaf, *Chem. Ber.*, **90**, 728 (1957).

(21) Ethyl 2-methoxypyrimidin-4-ylpyruvate, prepared by an identical procedure from 2-methoxy-4-methylpyrimidine, had m.p. 99.5–101.0°; $\lambda_{max}^{95\%\ EtOH}$ 378 mμ (ε 4400), 325 (12,900), 270 (4500), and 236 (3100); λ_{max}^{KBr} 5.78 and 6.14 μ; n.m.r. absorptions (measured in carbon tetrachloride) at τ 8.63 (triplet, J = 7 c.p.s.), 5.99, 5.73 (quartet, J = 7 c.p.s.), 3.71, 3.33 (doublet, J = 5 c.p.s.), 1.64 (doublet, J = 5 c.p.s.), and −3.27 (broad) of relative area 3:3:2:1:1:1:?. This spectrum resembled that reported for ethyl 3-methylquinoxal-2-ylpyruvate.[6] (*Anal.* Calcd. for $C_{10}H_{12}N_2O_4$: C, 53.57; H, 5.39. Found: C, 53.72; H, 4.91.) Ethyl pyrimidin-4-ylpyruvate, m.p. 134.5–136.5° (lit.[20] m.p. 135–136°), had $\lambda_{max}^{95\%\ EtOH}$ 384 mμ (ε 5200) and 313 mμ (ε 10,300); λ_{max}^{KBr} 2.94, 5.80, and 6.12 μ. It was observed that the intensity of the long wave-length absorptions at 378–384 mμ in the reference compounds, and at 400–420 mμ in product V, was markedly decreased when the spectra were measured in heptane.

(22) D. F. Elliott, A. T. Fuller, and C. R. Harington, *J. Chem. Soc.*; 85 (1948).

was heated briefly to effect solution and allowed to stand for several days. After addition of 26 ml. of water, four crops of crystals were obtained at −15° as follows: (a) 100 mg., m.p 130–144°; (b) 50 mg., m.p. 136–150°; (c) 100 mg., m.p. 110–160°; and (d) 60 mg., m.p. 160–180°. Recrystallization of fractions a and b from acetone gave a colorless solid, m.p. 135–185°, which showed two spots (t.l.c.)[19] at R_f 0.7 and 0.8.

Anal. Calcd. for $C_9H_{12}N_4O_3$ (VI, R = H): C, 48.21; H, 5.39. Calcd. for $C_{11}H_{14}N_4O_4$ (VI, R = Ac): C, 49.62; H, 5.30. Found (on different preparations): C, 48.01, 48.70; H, 5.52, 5.30.

Recrystallization of d from acetone gave a colorless solid, m.p. 182–185°; $\lambda_{max}^{95\%\ EtOH}$ 232 with shoulder at 264 mμ; $\lambda_{max}^{95\%\ EtOH}$ (pH 1) 278 and 232 mμ; λ_{max}^{KBr} 3.0–3.6 (broad), 3.16, 3.36, 5.80, and 6.05 μ; R_f (t.l.c.)[19] 0.8. This compound was presumed to be VI (R = Ac).

Other similar preparations of oximino ester VI gave variable yields of an apparently homogeneous product which by t.l.c. showed a single spot at R_f 0.7. After recrystallization from 95% ethanol the colorless solid had m.p. 148–149°; $\lambda_{max}^{95\%\ EtOH}$ 295 mμ (ε 4300) and 226 mμ (ε 20,000); $\lambda_{max}^{95\%\ EtOH}$ (pH 1) 301 and 220 mμ; λ_{max}^{KBr} 2.9–3.0 (broad), 3.01, 3.12, 3.61 (broad), 5.80, and 6.15 μ; n.m.r. (measured in hexadeuterioacetone) absorptions at τ 8.78 (triplet, J = 7 c.p.s.), 7.98 (multiplet), 6.10, 5.82 (quartet, J = 7 c.p.s.), 3.98, 3.55 (doublet, J = 5 c.p.s.), and 1.92 (doublet, J = 5 c.p.s.) with relative areas of 3:2:2:2:1:1:1. Addition of a singlet drop of deuterium oxide to the acetone solution caused immediate disappearance of the absorption at τ 3.98. This product was assumed to be VI (R = H).

β-(2-Aminopyrimidin-4-yl)alanine Hydrochloride.—The α-oximino ester VI was converted to *dl*-lathyrine by procedures similar to those described[22] previously. VIa–VIb mixture (100 mg.) was heated under reflux for 10 min. with 1 ml. of 1 *N* sodium hydroxide. After addition of 0.6 ml. of glacial acetic acid to the hot solution, colorless crystals slowly formed. The solid material was filtered and washed two times with water to afford 72 mg. of VII, m.p. 195–198° dec.; $\lambda_{max}^{95\%\ EtOH}$ (pH 1) 360 mμ (ε 300), 301 mμ (ε 5000), and 220 mμ (ε 27,000); λ_{max}^{KBr} 3.0–3.4 (broad), 3.54, 5.96, and 6.16 μ; R_f 0.65 (t.l.c. using 15:5:6 butanone–propionic acid–water).

To a solution containing 454 mg. (1.8 mmoles) of stannous chloride dihydrate in 2.2 ml. of concentrated hydrochloric acid was added 161 mg. (0.8 mmole) of VII. After standing several days, the reaction mixture was diluted with 25 ml. of water and saturated with hydrogen sulfide. The yellow precipitate formed was separated by filtration, and the filtrate was concentrated by lyophilization at 0° and 1 mm. to 151 mg. of a pale yellow solid, dec. >140°. The chromatographic behavior of this product was identical with that of natural lathyrine. The characteristic red color upon ninhydrin treatment was obtained, and identical R_f values were observed in the following solvent systems: 12:3:5 butanol–acetic acid–water (R_f 0.27, Whatman No. 1), 3:1 propanol–water (R_f 0.31, Whatman No. 1), 4:1 phenol–water containing 0.5% ammonia (R_f 0.53, silica gel G), and 15:5:6 butanone–propionic acid–water (R_f 0.23, silica gel G). The major peak observed during column chromatography (using a 50-cm. Amberlite IR-120 resin as absorbent, 0.38 *N* sodium citrate buffer at pH 4.26 as eluent, at 30–50°, in conjunction with a Beckman Spinco Model 120 amino acid analyzer) emerged at 6 hr., 12 min., with the optical density at 440 mμ 2.5 times greater than that at 570 mμ. An equimolar mixture of synthetic and natural lathyrine emerged from the column as a single symmetrical peak, with a D_{440}/D_{570} ratio of 2.5.

A portion (78 mg.) of lyophilized material was dissolved in 1 ml. of water, and acetone was added to a faint turbidity. Upon standing at −15°, 28 mg. of white solid (first crop) was obtained. After three recrystallizations (acetone–water) and drying (56°, 1 mm.), 8 mg. of colorless solid, dec. >165°, was obtained. This material had $\lambda_{max}^{pH\ 1}$ 298 and 222 mμ; $\lambda_{max}^{pH\ 13}$ 292 and 226 mμ; λ_{max}^{KBr} 2.9, 3.07, 2.9–4.4 (broad), 5.88, 6.11, and 6.29 μ.

Anal. Calcd. for $C_7H_{11}ClN_4O_2 \cdot 0.5H_2O$: C, 36.93; H, 5.31; Cl, 15.57; N, 24.62. Found: C, 37.66; H, 5.32; Cl, 15.44; N, 24.35.

When a drop of hydrochloric acid and additional acetone were added to the mother liquor from the first crystallization, 23.9 mg. of colorless needles, $[\alpha]^{27}D$ 0.0° (*c* 1.7, water) was obtained. This material, in contrast to that obtained in the first crop, possessed an infrared spectrum identical with that of natural lathyrine hydrochloride. This variation in infrared spectra is attributed to differences in crystal form.

Constituents of *Iva* Species. IV. Structure of Pseudoivalin, a New Guaianolide[1,2]

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Two new guaianolides, pseudoivalin and dihydropseudoivalin, have been isolated from the nonpolar sesquiterpene lactone fraction of a variety of *Iva microcephala* Nutt. Their structures are shown to be **2** and **3**. The stereochemistry **22** is postulated.

In an earlier paper[1] we described the isolation and structure determination of microcephalin (**1**), a polar sesquiterpene lactone constituent of a variety of *Iva microcephala* Nutt. collected in Taylor County, Florida.[3] We now deal with the nonpolar fraction which consists of two new sesquiterpene lactones, pseudoivalin (**2**) and its dihydro derivative **3**.

The two nonpolar constituents of *Iva microcephala* were difficult to separate. In fact the mixture, m.p 130–132°, had properties reminiscent of ivalin, m.p. 131–132°,[4] although it was finally resolved into a solid component (**2**), $C_{15}H_{20}O_3$, m.p. 122–123°, which was named pseudoivalin, and a liquid (**3**), $C_{15}H_{22}O_3$.[5]

The physical properties of pseudoivalin, $[\alpha]D$ −145°, high-intensity ultraviolet absorption at 210 mμ, infrared bands at 1760 and 1660 cm.$^{-1}$, suggested the presence of an exocyclic methylene group conjugated with a lactone function also found in other constituents of *Iva* species.[1,4,6] The presence of a second, perhaps tetrasubstituted double bond was inferred from a weak absorption near 1630 cm.$^{-1}$ in the infrared spectrum which also revealed the presence of a hydroxyl group. The latter was probably tertiary since it could not be acetylated with pyridine–acetic anhydride, although more vigorous conditions resulted in the formation of an acetate **4**.

The functional groups postulated on the basis of the spectroscopic properties were confirmed in the following way. Epoxidation of **2** and **4** resulted in the formation of two epoxides (**5** and **6**) whose ultraviolet (211 mμ, ε 7400) and infrared spectra (for **5**: 3700, 3500, 1770,

(1) Previous paper: W. Herz, G. Högenauer, and A. Romo de Vivar, *J. Org. Chem.*, **28**, 1700 (1964). In this paper the species yielding microcephalin was incorrectly attributed. The correct name is *Iva microcephala* Nutt.

(2) Supported in part by a grant from the United States Public Health Service (GM-05814).

(3) Material collected to the north and west of Tallahassee furnished ivalin consistently[1] and never any microcephalin or pseudoivalin.

(4) W. Herz and G. Högenauer, *J. Org. Chem.*, **27**, 905 (1962).

(5) That the mixture of solid **2** and liquid **3** melted at a temperature higher than that of the pure solid was an additional factor complicating analysis of the extract.

(6) W. Herz and N. Viswanathan, *J. Org. Chem.*, **29**, 1022 (1964).