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The Alkaloids of *Senecio mikanioides* Otto. Sarracine and Sarracine *N*-Oxide[1]

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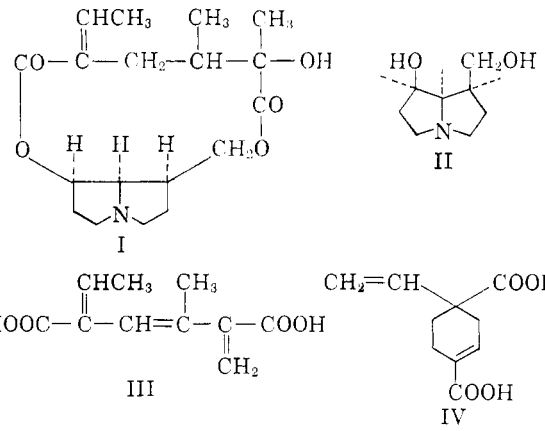
The major alkaloids in *Senecio mikanioides* Otto are sarracine and its *N*-oxide. Sarracine is shown to be platynecine esterified on the 1-hydroxymethyl group with 2-hydroxymethyl-2-butenoic acid, and on the 7-hydroxyl group with angelic acid. Angelic acid amide was also isolated from the plant, but this may be an artefact.

Present information on the toxicity of the pyrrolizidine alkaloids indicates that those which are esters of a saturated amino alcohol do not cause liver damage; but this view is based on the behavior of only one such alkaloid, platyphylline (I).[3,4]

Senecio mikanioides Otto was reported by Manske[5] to contain 0.02% of a noncrystallizable alkaloid which gave on hydrolysis a saturated amino alcohol subsequently identified[6] as platynecine (II), and a crystalline acid named "mikanecic acid." The common occurrence of this plant as a creeper on waste land near Melbourne prompted its reinvestigation with the aim of securing sufficient "mikanoidine" to determine whether it possessed hepatotoxic activity.

Adams and Gianturco[6] corrected the molecular formula of mikanecic acid to $C_{10}H_{12}O_4$, and, on the basis of spectral and hydrogenation data proposed the structure III. In a preliminary communication[7] we have shown that mikanecic acid is actually 1-vinyl-3-cyclohexene-1,4-dicarboxylic acid (IV). This acid is derived not by direct hydrolysis of the hypothetical "mikanoidine" but by condensation of two molecules of a C_5 acid.



I II III IV

An initial sample of *S. mikanioides* had 1.1% total alkaloid, of which 90% was *N*-oxide. Paper chromatography indicated that there was only one *N*-oxide, together with the corresponding tertiary base. The *N*-oxide crystallized readily from acetone, as a hydrate, $C_{18}H_7O_6N_2 \cdot H_2O$, m.p. 125-126°, which lost water only above 100° and regained it on standing in air. Reduction of the *N*-oxide with zinc dust-acid gave the tertiary base, $C_{18}H_7O_5N$, m.p. 45-46°, which was also isolated from the tertiary base fraction of the crude alkaloid. The *N*-oxide could be prepared from the tertiary base by hydrogen peroxide oxidation.

Physical properties and composition of the base and its *N*-oxide agree with those reported for the alkaloid sarracine and its *N*-oxide, isolated by

(1) Presented at I.U.P.A.C. Symposium on Natural Products, Melbourne, August, 1960.

(2) Chemical Research Laboratories, C.S.I.R.O., Melbourne, Australia.

(3) K. K. Chen, P. N. Harris, and C. L. Rose, *J. Pharmacol. Expt. Ther.*, **68**, 130 (1940).

(4) L. B. Bull and A. T. Dick, personal communcation.

(5) R. H. F. Manske, *Can. J. Res.*, **14B**, 6 (1936).

(6) R. Adams and M. Gianturco, *J. Am. Chem. Soc.*, **79**, 166 (1957).

(7) C. C. J. Culvenor and T. A. Geissman, *Chem. & Ind.*, 366 (1959).

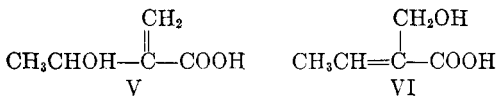
TABLE I

COMPARISON OF PROPERTIES OF BASES FROM *Senecio mikanioides* WITH SARRACINE AND SARRACINE *N*-OXIDE

Base, property	Danilova *et al.* (1953)	Present work
Sarracine		
Melting point	51–52°	45–46°
Specific rotation	−129.7°	−121°(ethanol)
Picrate m.p.	140–141°	141–142°
Bitartrate m.p.	177–179°	182–183°
Bitartrate spec. rotation	−70° (water)	−71° (water)
Sarracine *N*-oxide(hydrate)		
Melting point	123–124°	125–126°
Specific rotation	−81.6°	−94°(ethanol) −73°(water)
Picrate m.p.	107.7–108.5°	108–109°

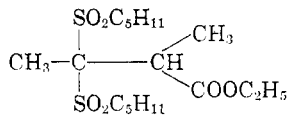
Danilova *et al.*[8] from *S. sarracenius*. The identity of the compounds from *S. mikanioides* and those from *S. sarracenius* was confirmed by the preparation of derivatives (Table I) and by the results of hydrolysis.

The Russian investigators hydrolyzed sarracine with dilute hydrochloric acid and obtained platynecine, angelic acid and an acid, $C_5H_8O_3$, called sarracinic acid. Sarracinic acid was an oil, characterized only by its zinc and silver salts.[9] It was shown to have one double bond and a hydroxyl group in an allylic position, subject to hydrogenolysis. Thus, on catalytic hydrogenation it gave 2-methylbutanoic acid. The two acids in sarracine esterify the two hydroxyl groups of platynecine. These observations were confirmed and led to either V or VI as possible structures for sarracinic acid.

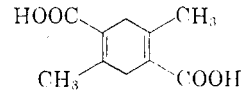


V VI

Catalytic hydrogenation of sarracine gave a mixture from which derivatives of tetrahydrosarracine and desoxytetrahydrosarracine have been obtained; but hydrolysis of the mixed product led only to angelic and 2-methylbutanoic acids. Hydrolysis of sarracine with 2.5*N* sodium hydroxide at 100° for an hour and a half gave a mixture of acids from which angelic acid, a crystalline acid, $C_5H_8O_3$, m.p. 56–57° and, in small amount, an acid, $C_{10}H_{12}O_4$, m.p. 240°, were isolated. Increasing the period of hydrolysis to twenty-four hours caused the yield of the C_{10} acid to increase to 50%, while the acid $C_5H_8O_3$ could no longer be isolated. Slow conversion of the later acid into the former under these conditions was readily demonstrated. As reported earlier,[7] the C_{10} acid is the acid isolated by Manske[5] and named mikanecic acid. It has the structure IV, and is evidently the end product of reactions which would be expected to give 1,3-butadiene-2-carboxylic acid. For example, the nickel carbonyl carbonylation of vinylacetylene,[10] and the pyrolysis of methyl 2-acetoxy-2-methyl-3-butenoate[11] yield the acid IV. The dicarboxylic acid, $C_{10}H_{12}O_4$, m.p. 234°, obtained by Posner[12] from the reaction of the disulfone VII with alkali, and formulated as VIII, is undoubtedly IV.

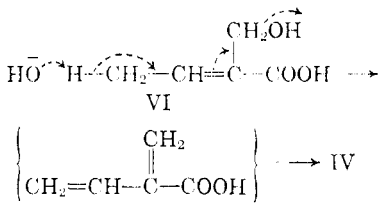


VII



VIII

Although it is not possible to establish the identity of the poorly characterized, noncrystalline acid of Danilova and Kusovkov with the acid with m.p. 56–57°, it is proposed to call the latter sarracinic acid since it is the acid moiety actually present in the natural alkaloid (see below). In the present work, acid hydrolysis of sarracine gave, besides angelic acid, a noncrystallizable acid of the same R_f as the crystalline acid, and possibly a mixture. The conversion of sarracinic acid into mikanecic acid is undoubtedly a reaction of the acid VI:



VI

That sarracinic acid is actually VI, and not V which is isomerized to VI in the course of conversion to IV, is borne out by the properties of a synthetic ester of V.[13] The acetate of V, prepared by the nickel carbonyl carboxylation of 3-acetoxy-1-butyne, when heated with alkali at 100° under conditions that converted sarracinic acid into "mikanecic acid," yielded no mikanecic acid. Thus, the acid V is not converted into VI under these conditions.

Ozonolysis of sarracinic acid gave acetaldehyde but no trace of formaldehyde. This is strong evidence for structure VI, but its value was somewhat lessened by the formation also of some pyruvic acid in the ozonization, an indication that some rearrangement had occurred. Attempts to hydrogenate sarracinic acid were unsuccessful: The

(8) A. V. Danilova, R. Konovalova, P. Massagetov, and M. Garina, *J. Chem. V.S.S.R.*, **23** (85), 1417 (1953).

(9) A. V. Danilova and A. Kusovkov, *J. Chem. V.S.S.R.* **23** (85), 1597 (1953).

(10) A. Yakubovich and E. V. Volkova, *Doklady Akad. Nauk S.S.S.R.*, **84**, 1183 (1952).

(11) E. A. Brande and E. A. Evans, J. Chem. Soc., 3238 (1956).

(12) T. Posner, *Ber.*, **34**, 2643 (1901).

(13) C. C. J. Culvenor and T. A. Geissman, *J. Am. Chem. Soc.*, **83**, 1647 (1961). A sample of this acid was first prepared by Dr. M. C. Whiting for the experiments described in the present paper.

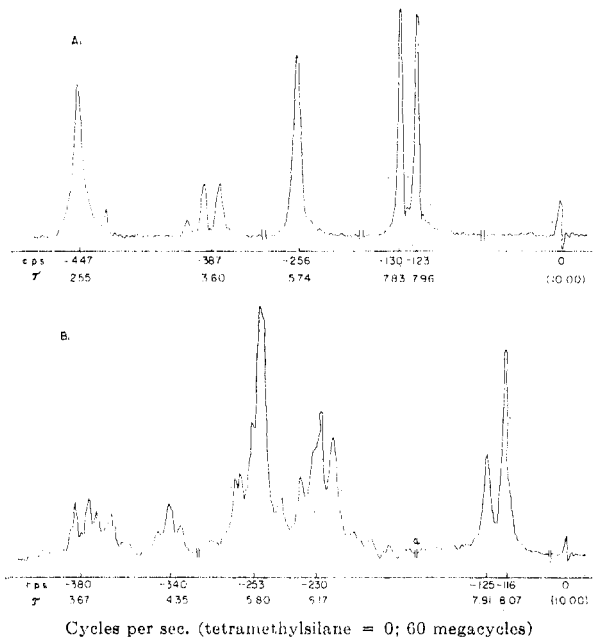


Fig. 1. NMR spectra (solution in deuterochloroform): A, sarracinic acid; B, sarracine *N*-oxide. Vertical scale changes at a

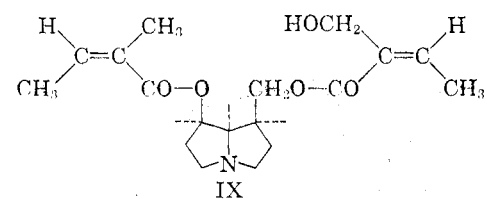
hydroxyl group was hydrogenolyzed and only 2-methylbutanoic acid could be isolated. Attempts to form crystalline derivatives of sarracinic acid also failed; attempted acetylation with acetic anhydride and pyridine at room temperature gave mikanecic acid.

Proof that sarracinic acid is indeed VI was at length provided by the NMR spectrum (Fig. 1). This shows only one proton on a doubly bonded carbon atom; its signal, centered at 385 cps (relative to internal tetramethyl silane, at 60 mc.), is a quadruplet as required for the grouping CH_3—CH=C. The doublet at 123, 130 c.p.s. is due to the CH_3 protons spin coupled with the adjacent olefinic proton, and the signal at 256 c.p.s. is that of the CH_2 group. This spectrum is compatible with structure VI but not with V.

There remained the possibility that sarracine was an ester of angelic acid and V (not VI), and that during alkaline hydrolysis rearrangement of V to VI occurred (although the experiment described above, using the acetate of V, made this unlikely). The NMR spectrum of sarracine *N*-oxide was compatible with IX, containing VI as the acid moiety. From area measurements, the signal groups at −370 c.p.s. and −338 c.p.s. must represent two protons and one proton, respectively. The latter is temperature dependent, moving down field with a rise in temperature, and thus is due to the hydroxylic proton. Its triplet nature is characteristic of the —CH_2OH group of structure IX, whereas the —CHOH group of the alternative structure would give a doublet. The signals centered at −370 cps, because of protons on doubly bonded carbon atoms, are composed of two quadruplets in almost identical positions, as would be expected for the two CH_3—CH=C< groups of structure IX. If the alkaloid were an ester of V, only one such group would be present, along with a —C(=)=CH_2 group, which should produce a clearly distinguishable signal. The methyl group signals at −116 and −123 cps are those of three methyl groups attached to doubly bonded carbon; one should give a singlet and the other two doublets in essentially the same position. These signals are in agreement with structure IX; the alternative structure, having a CH_3CHOH grouping, should show a methyl group signal further upfield.

Sarracine is thus a diester of platynecine with angelic and sarracinic acid (VI). To establish the respective points of attachment of the two acid residues, controlled alkaline hydrolysis under mild conditions was carried out, with identification of the liberated acids by paper chromatography. Sarracinic acid was liberated at a much faster rate than angelic acid. As the acids differ only in the presence of a β-hydroxyl group in sarracinic acid, too far removed to exert a large inductive influence on the rate of hydrolysis of the ester, this is evidence that sarracinic acid is attached to the primary hydroxyl group of the base.

The remaining structural detail is the configuration of sarracinic acid about the double bond. The answer to this question was readily found with the aid of the information recently published by Nair and Adams,[14] who have related the chemical shift of a series of *cis-trans*-isomeric acids to the relative position of the proton β- to the carboxyl group in α,β unsaturated acids and esters. The τ values of the acids related to tiglic (H and COOH *cis*) and those related to angelic (H and COOH *trans*) were found to differ by about 0.7, the latter having τ values ranging (for eight compounds) from 3.70 to 4.03. The τ value for the olefinic proton of sarracinic acid is found to be 3.6 to 3.8 (depending upon whether the value is read from the spectrum of the acid or the alkaloid) which established its configuration as shown in the complete stereochemical representation of sarracine (IX):



IX

It is of interest that the stereochemistry of angelic and sarracinic acids is the same. The possibility that sarracinic acid is a direct biological oxidation product of angelic acid is suggested by this observation. It may also be noted that angelic

(14) M. D. Nair and R. Adams, *J. Am. Chem. Soc.*, 82, 3786 (1960).

acid is isoprenoid in its carbon skeleton, but that the carbonyl group is in a position corresponding to the methyl group of the precursor that may be regarded as immediately derived from mevalonic acid (*cf.*, senecioic (β-methylcrotonic)acid), and the terminal methyl group represents the carboxyl (or hydroxymethyl group) of such a precursor. The interesting possibility that angelic acid represents a rearrangement, and not an oxidation-reduction, product derived from senecioic acid, is under study.

An acid called plantenolic acid, to which structure VI was assigned, has been isolated from the seeds *Plantago major* var. *asiaticum* by Ogata and Nishioji.[15] Like sarracinic acid, it reduced Tollen's reagent and gave a positive iodoform test. The identity of plantenolic acid and sarracinic acid is possible but cannot be assumed in the absence of direct comparison.

From one sample of crude alkaloid from *S. mikanioides* was isolated a neutral compound, C_5H_9ON. This was identified as angelic acid amide by comparison with an authentic specimen. Angelic acid amide was previously prepared by Bruylants[16] by hydrolysis of the *cis*-isomer of α-methylcrotononitrile. As ammonia was used during the isolation of the alkaloids it is possible that it is an artefact.

EXPERIMENTAL

(a) *Extraction of Senecio mikanioides.* Dried plant (28 lbs.) from Brighton Beach, Victoria, was milled and extracted with methanol (Soxhlet). After removal of the solvent the residue was extracted with dilute sulfuric acid and the aqueous solution was made alkaline with sodium hydroxide, saturated with salt, and extracted with chloroform in fifteen portions. The first five extracts gave 200 g. of greenish-black oil, the second 38 g., and the third 18 g. of dark gum.

The 200-g. portion was re-extracted into 0.5*M* sulfuric acid and the aqueous solution made alkaline with ammonia and extracted with three lots of five portions each of chloroform. All of the chloroform extracts yielded crystalline sarracine *N*-oxide; total yield 72 g.

Recrystallized from acetone, sarracine *N*-oxide has m.p. 125–126°, $[\alpha]_D^{22}$ −94° (*c*, 2.01 in ethanol), $[\alpha]_D^{19}$ −73° (*c*, 2.14 in water).

Anal. Calcd. for $C_{18}H_{27}O_6N \cdot H_2O$: C, 58.12; H, 7.8; N, 3.8; H_2O, 5.3. Found: C, 58.3; H, 7.7; N, 3.9; loss on drying (100°, vac., phosphorus pentoxide), 5.1%.

Analyses of the dried sample were unsatisfactory, probably because of the rapidity with which water is regained. A sample of sarracine *N*-oxide heated at 110° for 30 min. melted sharply at 140–141°.

Anal. Calcd. for $C_{18}H_{27}O_6N$: C, 61.1; H, 7.7; N, 4.0. Found: after drying, C, 60.1; H, 7.8; N, 3.9.

When this sample was kept in the air for 10 min. its m.p. returned to 125–126°.

The crude alkaloid remaining after crystallization of sarracine *N*-oxide (above) was divided into tertiary base and *N*-oxide fractions by taking up in dilute acid, making basic, and extracting first with benzene (fraction A) and then with chloroform (fraction B). Fraction A showed a single spot, R_f 0.62 (butanol-5% acetic acid) and showed no sign of inhomogeneity when chromatographed on alumina or on glass powder bearing *p*H 7 phosphate buffer. It could not be made to crystallize but when oxidized with hydrogen peroxide, formed an *N*-oxide, m.p. 123–124°, R_f 0.70, $[\alpha]_D^{22}$ −91° (*c*, 1.79 in ethanol), mixed m.p. with sarracine *N*-oxide, 124–125°. Seeding with sarracine obtained by reduction of the *N*-oxide caused crystallization of one fraction and after rubbing on a porous tile this material had m.p. 41–43°, mixed m.p. 42–44°.

(b) *Picrate of sarracine N-oxide.* The addition of an aqueous solution of picric acid to sarracine *N*-oxide in water solution yielded the picrate. Recrystallized from aqueous methanol, then from water, it melted at 108–109° (reported by Danilova *et al.*,[8] m.p. 107.5–108.5°).

Anal. Calcd. for $C_{18}H_{27}O_6N \cdot C_6H_3O_7N_3$: C, 49.5; H, 5.2; 3 × C—CH_3, 7.7. Found: C, 49.8; H, 5.3; C—CH_3, 5.1.

(c) *Sarracine.* To a stirred solution of 5.0 g. of sarracine *N*-oxide in 100 ml. of 2*N* sulfuric acid was added zinc dust in small portions over a period of 2 hr. The filtered solution was made alkaline with ammonia and extracted with chloroform. Removal of the chloroform left a nearly colorless oil which crystallized on standing, m.p. 45–46°, $[\alpha]_D^{17}$ −121° (*c*, 1.09 in ethanol) (reported by Danilova *et al.*,[8] m.p. 51–52°, $[\alpha]_D$ −129.7°).

(d) *Sarracine picrate*, prepared in methanol solution and recrystallized from ethyl acetate-petroleum ether (b.p. 40–60°), formed shining yellow leaflets, m.p. 141–142° (reported by Danilova *et al.*,[8] m.p. 140–141°).

Anal. Calcd. for $C_{18}H_{27}O_5N \cdot C_6H_3O_7N_3$: C, 50.9; H, 5.4; N, 9.9; 3 × C—CH_3, 7.9. Found: C, 51.3; H, 5.4; N, 9.7; C—CH_3, 5.7.

(e) Sarracine bitartrate crystallized from ethanol or aqueous ethanol in glistening plates, m.p. 182–183°, $[\alpha]_D^{23}$ −71° (*c*, 1.98 in water).

Anal. Calcd. for $C_{22}H_{33}O_{11}N$: C, 54.2; H, 6.8; N, 2.9. Found: C, 54.1; H, 6.9; N, 3.0.

Danilova *et al.*[8] reported m.p. 177–179°, $[\alpha]_D$ −70° (water).

(f) *Alkaline hydrolysis of sarracine* (i). The sarracine obtained from the reduction with zinc of 5.0 g. of the *N*-oxide was refluxed for 2 hr. with a solution of 5 g. of potassium hydroxide in 25 ml. of aqueous methanol. The methanol was removed under reduced pressure and the residue acidified and extracted with ether. The residue left after removal of the ether was steam-distilled, affording 1.28 g. of angelic acid, m.p. 43–44° (undepressed on admixture with authentic material). The nonsteam-volatile residue, after ether extraction, was a colorless viscous oil (0.4 g.) which could not be crystallized. When treated with iodine and sodium hydroxide solution, iodoform, m.p. 119–121°, was formed. The acid quickly reduced an alkaline Tollen's reagent.

The aqueous solution resulting from the saponification, after removal of the acids, was made strongly alkaline and extracted continuously with ether. Removal of the ether left an oily residue which crystallized when seeded with platynecine. Recrystallized from acetone, it had m.p. 145–146°; mixed with authentic platynecine, m.p. 146–147°, the melting point was 145.5–146.5°.

In another experiment, in which the solution after saponification of sarracine was made strongly acidic and steam distilled, recovery of the basic fraction gave *anhydroplatynecine*, identified as the picrate, m.p. 265–270° dec. (reported,[17] m.p. 265–270° dec.).

(ii) Sarracine *N*-oxide (3 g.) was heated at 100° for 2 hr. in 2.5*N* sodium hydroxide (50 ml.). The mixture was cooled, acidified to Congo Red with sulfuric acid, extracted with three lots of petroleum ether (b.p. 40–60°) (extract A), then with six lots of ether (extract B), and finally continuously with ether for 24 hr. (extract C). The acid fractions obtained were as follows:

(15) A. Ogata and R. Nishioji, *J. Pharm. Soc., Japan*, **1924**, 1040.

(16) P. Bruylants, L. Ernould, and M. Dekoker, *Bull. Acad. Roy. Belg.*, **16**, 721 (1930).

(17) A. Orechov and R. Konovalova, *Ber.*, **68**, 1886 (1935).

Fraction	Weight, G.	R_f(C_4H_9OH—AcOH)	R_f(C_4H_9OH—NH_3)
A	0.80	—	0.30
B	0.83	0.87, 0.76	0.13, 0.04
C	0.15	0.76	0.13, 0.04
	Angelic acid	—	0.27
	Sarracinic acid	0.76	0.13
	Mikanecic acid	0.87	0.04

Fraction A is angelic acid, R_f 0.27 (butanol-ammonia). A small amount of crystals which formed in fraction B were readily filtered after addition of water; crystallization from water gave mikanecic acid, m.p. 240°.

Anal. Calcd. for $C_{10}H_{12}O_4$ (dibasic): C, 61.2; H, 6.2; equiv. wt. 98. Found: C, 61.4; H, 6.3; equiv. wt. 92.

A mixture with authentic 1-vinyl-3-cyclohexene-1,4-dicarboxylic acid was undepressed. Mikanecic acid has R_f 0.87 in butanol-acetic acid, 0.04 in butanol-ammonia. The main part of fraction B was evaporated to dryness, taken up in hot benzene, a portion of the solution distilled to remove any residual water, and then cooled to give *sarracinic acid* as long needles, m.p. 57–58°.

Anal. Calcd. for $C_5H_8O_3$: C, 51.7; H, 6.9; O, 41.3; (C)CH_3 12.9; equiv. wt. 116. Found: C, 52.0; H, 6.9; O, 40.8; (C)-CH_3, 12.1; equiv. wt. 117.

Sarracinic acid is not hygroscopic and is optically inactive. It may be extracted completely from aqueous solution by batchwise ether extraction if the solution is saturated with salt. Fraction C was shown to be also a mixture of sarracinic and mikanecic acids.

(iii) Sarracine *N*-oxide (0.5 g.) and 2.5*N* sodium hydroxide (15 ml.) were heated at 100° for 24 hr. in a polythene flask. The solution was cooled and acidified and the precipitate filtered to give mikanecic acid (92 mg.; 54%) m.p. 233–234°.

(g) *Acid hydrolysis of sarracine N-oxide.* Sarracine *N*-oxide (1.7 g.) was heated at 100° in 10% hydrochloric acid for 4 hr. Extraction with petroleum ether (b.p. 40–60°) gave angelic acid (0.17 g.). Further extraction with ether gave an oil (0.54 g.) R_f 0.73 (butanol–acetic acid) which was taken up in benzene. The solution was boiled to remove water, cooled, and seeded with sarracinic acid. The acid precipitated only as an oil.

(h) *Conversion of sarracinic acid into mikanecic acid.* Sarracinic acid (0.2 g.) was heated at 100° in 2.5*N* sodium hydroxide (20 ml.) and samples (1 ml.) withdrawn at intervals, acidified, saturated with salt, and extracted with ether. The extracts were evaporated, made up to 0.5 ml., and spotted on a paper chromatogram. The chromatogram indicated about 10% sarracinic acid, 90% mikanecic acid after 16 hr., and essentially all mikanecic acid after 24 hr. The remaining solution was similarly worked up after 24 hr. to give mikanecic acid, m.p. 234°.

(i) *Partial hydrolysis of sarracine.* To a solution of 0.96 g of sarracine in water containing 2.50 ml. of 1.09*N* sulfuric acid were added 15 ml. of ethylene glycol and 10.6 ml. of 0.51*N* sodium hydroxide, and the solution was made up to 50.0 ml. Ten-milliliter aliquots were removed (a) at once, and then at intervals of (b) 2 hr., at room-temperature, (c) after 15 min. at 64°, (d) 30 min. later, and (e) 30 min. later, both at 64°. Each aliquot was acidified, extracted three times with ether, and the ether solutions shaken with sodium sulfate and evaporated after the addition of a few drops of ammonia. The final solutions were chromatographed in butanol saturated with 2*N* ammonia. Aliquot (b) showed sarracinic acid as a faint spot, but no angelic acid, aliquot (c) showed a strong sarracinic acid spot and a faint trace of angelic acid. Both (d) and (e) showed both acids and (a) contained none.

(j) *Hydrogenation of sarracine and sarracine N-oxide.* When reduced with hydrogen and platinum oxide in ethanol or ethyl acetate sarracine took up 2 moles, sarracine *N*-oxide 3 moles of hydrogen. *Tetrahydrosarracine* was an oil that could not be crystallized, but it formed a picrate which crystallized from methanol-water as soft, yellow needles, m.p. 70–71°.

Anal. Calcd. for $C_{18}H_{31}O_5N \cdot C_6H_3O_7N_3$: C, 50.5; H, 6.0; N, 9.8; 3 × CH_3, 7.9. Calcd. for $C_{18}H_{31}O_4N \cdot C_6H_3O_7N_3$: C, 52.0; H, 6.2; N, 10.1. Found: C, 51.1, 51.2; H, 5.8, 5.9; N, 9.9; C—CH_3, 6.5.

(k) *Hydrolysis of tetrahydrosarracine.* After the reduction of 5.0 g. of sarracine *N*-oxide with zinc–sulfuric acid the recovered sarracine was reduced in ethanol solution with hydrogen (1 atm.) and Adams' catalyst. After removal of the solvent a sample was removed for conversion to the picrate and the residual material was saponified by 2 hr. heating with a solution of 5.0 g. of potassium hydroxide in 50 ml. of 50% aqueous methanol. The solution was acidified and steam distilled. The distillate was neutralized (to phenolphthalein) and treated with *p*-phenylphenacyl bromide, to yield 4.1 g. of *p*-phenylphenacyl esters, m.p. 65–75°. A portion of this crystallized from petroleum ether (b.p. 40–60°), and after recrystallization from benzene–petroleum ether had m.p. 87–88°.

Anal. Calcd. for angelic *p*-phenylphenacyl ester, C_{19}-$H_{18}O_3$: C, 77.6; H, 6.2; 2 × CCH_3, 10.2 Found: C, 77.4; H, 6.3; C—CH_3, 8.8.

Mixed with an authentic sample of the *p*-phenylphenacyl ester of angelic acid (m.p. 88–89°) the m.p. was 87–89°.

The petroleum ether–soluble material was chromatographed on alumina. Separation, even with petroleum ether (b.p. 40–60°) as the eluant, was poor and incomplete, but the first fractions gave crystalline material, m.p. 66–67°, which did not depress the m.p. of authentic α-methylbutyric *p*-phenylphenacyl ester, m.p. 70–71°.

Anal. Calcd. for α-methylbutyric *p*-phenylphenacyl ester, $C_{19}H_{20}O_3$: C, 77.0; H, 6.8; 2 × C—CH_3, 10.1. Found, ester m.p. 66–67°: C, 77.1; H, 6.7; C—CH_3, 7.9.

(l) *Desoxytetrahydrosarracine N-oxide picrate.* After the hydrogenation of a 3-mmole sample of sarracine the oily tetrahydro base was treated with 3 ml. of hydrogen peroxide (130 vol.) in 10 ml. of ethanol. After 18 hr., the hydrogen peroxide was destroyed with manganese dioxide and the solvent removed. Addition of picric acid in methanol resulted in the formation of a picrate which, after recrystallization from water, had m.p. 127–128°. This appears to be the picrate of desoxytetrahydrosarracine *N*-oxide.

Anal. Calcd. for $C_{18}H_{31}O_5N \cdot C_6H_3N_3O_7$: C, 50.5; H, 6.0; 3 × C—CH_3. 7.9; 4 × C—CH_3, 10.5. Found: C, 50.2; H, 6.0; C—CH_3, 7.0.

(m) *Ozonolysis of sarracinic acid.* Ozone was passed through a solution of crystalline sarracinic acid (0.24 g.) dissolved in 0.5*N* sulfuric acid (50 ml.) for 3 hr. The resulting solution was shaken with hydrogen and platinum catalyst until hydrogen was no longer absorbed and then divided into two equal portions. One portion was heated in a stream of air which passed into dimedon solution. No precipitate formed in the dimedon after leaving overnight but when the solution was concentrated, the distillate gave a precipitate of acetaldehyde dinitrophenylhydrazone (40 mg.) m.p. and mixed m.p. 159–160°, when added to aqueous 2,4-dinitrophenylhydrazine hydrochloride. The concentrated dimedon solution gave crystals (83 mg.), m.p. 140–141°, mixed m.p. 141–142° with authentic dimedon derivative of acetaldehyde. The second portion of the ozonolysis solution was heated and the volatile materials led into 2,4-dinitrophenylhydrazine solution, to give acetaldehyde dinitrophenylhydrazone (66.5 mg.), m.p. 158–159°, mixed m.p. 159–160°. The non-volatile residue was treated with dinitrophenylhydrazine hydrochloride and the precipitate (102 mg.) filtered and crystallized from methanol to give pyruvic acid dinitrophenylhydrazone, m.p. 215° dec. undepressed on admixture with authentic material.

Anal. Calcd. for $C_9H_8O_6N_4$: C, 40.3; H, 3.0; O, 35.8; N, 20.9. Found: C, 40.9; H, 3.1; O, 34.2; N, 20.6.

The paper chromatographic system of Gasparic and Ve-

cera[18] (paper impregnated with dimethylformamide and developed with cyclohexane), was used to show conclusively that the initial precipitates of acetaldehyde dinitrophenylhydrazone and their formation solutions contained no trace of formaldehyde dinitrophenylhydrazone.

(n) *Ozonolysis of sarracine N-oxide.* Sarracine *N*-oxide (1.0 g.) was ozonized by the method used for sarracinic acid (II, m). Only acetaldehyde dinitrophenylhydrazone (253 mg; 0.8 mole) was isolated.

(o) *Hydrogenation of sarracinic acid.* Sarracinic acid (0.25 g.) was shaken with hydrogen and a weak Raney nickel catalyst in aqueous solution until 1 mole of hydrogen (48 ml.) was absorbed. The catalyst was removed and the solution extracted with petroleum ether (b.p. 40–60°) (extract A), saturated with sodium chloride and re-extracted with ether (extract B). Extract A gave an oil (0.09 g.) R_f 0.45 in butanol-ammonia, no spot in butanol-acetic acid (angelic acid R_f 0.41, no spot, resp.), which was converted into the *p*-phenylphenacyl ester of α-methylbutyric acid, m.p. and mixed m.p. 68–69°. Extract B gave an oil (0.13 g.), R_f 0.25 in butanol-ammonia, 0.70 in butanol-acetic acid, as expected for a hydroxylated C_5 acid (sarracinic acid, R_f 0.25, 0.70 resp.). However, no crystalline derivative could be obtained with phenyl isocyanate or with *p*-phenylphenacyl bromide. A similar result was obtained after hydrogenation with a platinum catalyst.

(p) *Action of acetic anhydride on sarracinic acid.* Acetic anhydride (0.2 g.) was added to a solution of sarracinic acid (150 mg.) in pyridine (0.8 ml.) at 0°. After 36 hr. at room temp. the mixture was poured into dilute sulfuric acid and extracted with ether to give a mixture of oil and solid. Rubbing with chloroform permitted the solid to be filtered. This was mikanecic acid (29 mg.), m.p. and mixed m.p. 238–239. The chloroform solution was extracted with dilute sodium hydroxide, the extract washed with chloroform and then acidified and re-extracted with chloroform, then with ether. The chloroform extract gave an oily solid (10–15 mg.) showing a spot on a paper chromatogram only for mikanecic acid, and the ether extract gave essentially pure mikanecic acid (24 mg.), m.p. 231°.

(q) *Action of sodium hydroxide on 3-acetoxy-2-methylenebutanoic acid.* 3-Acetoxy-2-methylenebutanoic acid (0.2 g.)[13] and 1*N* sodium hydroxide (15 ml.) were heated at 100° in a polythene flask for 24 hr. The solution was cooled, acidified (no precipitate formed), saturated with salt and extracted with ether to give a gum (0.15 g.) R_f 0.83 in butanol-acetic acid, 0.16 in butanol-ammonia (sarracinic acid, R_f 0.80, 0.15 resp., on same sheet). Mikanecic acid was therefore absent. Hydrolysis of 3-acetoxy-2-methylenebutanoic acid with 0.5*N* sodium hydroxide at room temperature for several hours gave a product with the same R_f values.

(r) *Isolation of angelic acid amide.* The alkaloid (72 g.) called fraction B in section II(a) was chromatographed on alumina. Two fractions (total, 0.2 g.) eluted by chloroform contained crystals which were separated from amorphous material by extraction with hot petroleum ether (b.p. 40–60°). Recrystallization from this solvent gave long needles, m.p. 128°.

Anal. Calcd. for C_5H_9ON: C, 60.6; H, 9.1; N, 14.1. Found: C, 60.6; H, 9.0; N, 14.0.

The compound was readily soluble in organic solvents and fairly soluble in water, giving a solution neutral to litmus. Admixture with authenic angelic acid amide, described below, caused no depression of the melting point.

When sarracine was kept at 50° for 4 days in 2*N* aqueous ammonia, the alkaloid was completely hydrolyzed but no angelic acid amide could be isolated from the solution.

(s) *Preparation of angelic acid amide from angelic acid.* Angelic acid (0.5 g.) was added to thionyl chloride (5 ml.) at 0°. After 1 hr. excess thionyl chloride was removed at room temperature under reduced pressure. The residue was taken up in dry ether (2 ml.) and added drop-wise to cooled aqueous ammonia (10 ml.), stirred, and kept below room temperature. After 30 min., the mixture was diluted with water and extracted with chloroform to give a solid (0.23 g.) which after purification on alumina and crystallization from petroleum ether (b.p. 40–60°), had m.p. 128° (Found: C, 60.4; H, 9.0; N, 14.1). Another preparation, in which the flask was warmed slightly during evaporation of the excess thionyl chloride gave a product, m.p. 68–72°, mainly tiglic acid amide. It was evident also that angelic acid amide is partially converted into tiglic acid amide if refluxing in petroleum ether (b.p. 40–60°) is prolonged during recrystallization.

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