ketones and aldehydes were obtained from commercial sources and were used without purification. Reagent or C.P. grades were used when available. U.S.P. flowers of sulfur and Karl Fischer reagent grade pyridine were used.

Preparation of 1-Phenyl-4-thiobenzoylpiperazine.—Sulfur (3.2 g.) was placed in a 200-ml. flask and pyridine was added until the flask was about one-third full. The flask was swirled gently as 10.6 g. (10.1 ml.) of benzaldehyde and 16.2 g. (16.0 ml.) of N-phenylpiperazine were added. The flask was filled to three-fourths of its capacity with pyridine, attached immediately to a condenser, and held at reflux temperature for 2 hours; yield 83%, m.p. 93.5–94.5°.

Purification of Thioaroyl Derivatives.—The hot reaction mixture was diluted with 400–500 ml. of 95% ethanol and allowed to cool. The product was collected and washed with ethanol. Repeated recrystallizations from ethanol, with decolorizing charcoal, gave analytically pure samples.

Those compounds having very limited solubilities in ethanol were dissolved in an acetone–ethanol mixture.

Purification of Thioalkanoyl Derivatives.—After the pyridine was removed by steam distillation, the non-aqueous layer was extracted with boiling hexane and decolorized with charcoal. The solution was cooled in a Dry Ice-bath, and the crystals were filtered off. They were further purified by recrystallizations from ethanol or ethanol–water mixtures.

Description of the Compounds.—These compounds range in color from a deep orange through all shades of yellow to a very pale ivory; the aromatic derivatives are generally much more strongly colored than the aliphatic derivatives. Some of the compounds are very lustrous.

Physical and analytical data on the compounds prepared during this research are given in Table I. Yields probably reflect difficulty of purification rather than degree of reaction. Analytical work was done by Geller Microanalytical Laboratories, Hackensack, N. J.

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Alkaloid Studies. IX.[1] Rauwolfia Alkaloids. IV.[2] Isolation of Reserpine and Other Alkaloids from Rauwolfia sellowii Muell. Argov.[3]

BY S. C. PAKRASHI,[4a] CARL DJERASSI,[4a] RICHARD WASICKY[4b] AND N. NEUSS[4c]

RECEIVED AUGUST 17, 1955

The recent interest in different *Rauwolfia* species[5] as possible sources for reserpine and other new alkaloids of this type led us to a chemical investigation of the brazilian tree, *Rauwolfia sellowii* Muell. Argov. This species has been characterized histochemically[6] and an alkaloidal fraction derived from it has been shown to possess hypotensive activity with an indication of an action in the hypothalamic region.[7] More recently,[8] the isolation of ajmaline, ajmalinine, serpentine and two unidentified crystalline alkaloids has been reported. However, these alkaloids were neither fully characterized nor compared with authentic specimens while the most active fraction remained in an amorphous form.

By a procedure, based in part upon that employed by Hochstein, *et al.*,[9] in their investigation of *R. heterophylla*, and outlined in the Experimental portion, we have been able to isolate seven alkaloids. While all but one are known, the relative proportions and, in particular, the co-occurrence of several of them in the same plant is of some significance.

Quantitatively, the principal alkaloids of the root bark of *R. sellowii* proved to be ajmaline[10] (1.2%) and aricine[11] (1.5%), thus making this plant by far the most abundant source for the former. Of considerable biogenetic interest is the fact that both alkaloids are accompanied in trace amounts by closely related bases. In addition to aricin, there has been encountered 0.0009% of ajmalicine,[12] which from a structural standpoint may be considered to be the parent compound of this class, and 0.0056% of its stereoisomer, py-tetrahydroalstonine.[13] This represents the first isolation of py-tetrahydroalstonine from a *Rauwolfia* species.

Two dihydroindoles of unknown constitution appear to belong to the ajmaline group. The first, tetraphyllicine, has been isolated recently[5b] from *R. tetraphylla* and is the first oxygen-free ($C_{20}H_{26}N_2$) *Rauwolfia* alkaloid. The remarkable similarity of its physical constants with those of ajmaline have been mentioned already[5b] and it was suggested at that time that tetraphyllicine may conceivably be the oxygen-free parent substance of the ajmaline group. This suggestion is now somewhat strengthened by the observed coexistence of the two alkaloids in *R. sellowii;* ajmaline had not been found in *R. tetraphylla*.[5b] The other alkaloid, m.p. 241–242°, now named ajmalidine, appears to be new and is formulated tentatively as $C_{20}H_{24}N_2O_2$. Lack of material prevented degradative experiments but its physical properties strongly suggest a close relationship to ajmaline ($C_{20}H_{26}N_2O_2$). The color reaction with nitric acid and the ultraviolet absorption spectrum are practically identical with those of ajmaline. The infrared spectrum of ajmalidine resembles that of ajmaline except for the presence of a pronounced band at 5.77 μ ($CHCl_3$), which on the basis of its position and molar intensity can be assigned to a five-membered ring ketone.

(1) For paper VIII in the Wayne series "Alkaloid Studies" see C. Djerassi, C. R. Smith, A. E. Lippman, S. K. Figdor and J. Herran, THIS JOURNAL, **77**, 4801 (1955).

(2) For paper III in the Lilly series "Rauwolfia Alkaloids" see N. Neuss, *et al.*, *ibid.*, **77**, 4087 (1955).

(3) An investigation of the alkaloids of *R. sellowii* was started independently in the Lilly Research Laboratories and as part of a coöperative Wayne University–Universidade de São Paulo research project. Upon learning of each other's results, it was decided to publish the work as a joint contribution from the three laboratories. Reprint requests should be addressed to N. Neuss, Eli Lilly and Co., Indianapolis, Ind.

(4) (a) Wayne University; (b) Universidade de São Paulo; (c) Eli Lilly and Co.

(5) *Cf.* (a) A. Chatterjee and S. Talapatra, *Naturwiss.*, **42**, 182 (1955); (b) C. Djerassi and J. Fishman, *Chemistry and Industry*, 627 (1955), and references cited therein.

(6) Th. A. Neubern de Toledo and Robert Wasicky, *Scientia Pharm.*, **22**, 217 (1954).

(7) R. A. Seba, J. S. Campos and J. G. Kuhlmann, *Rev. quim. farm.*, **19**, 11 (1954).

(8) R. A. Seba, J. S. Campos and J. G. Kuhlmann, *Bol. inst. vital Brazil*, **5**, 175 (1954).

(9) F. A. Hochstein, K. Murai and W. H. Boegemann, THIS JOURNAL, **77**, 3551 (1955).

(10) F. C. Finch, J. D. Hobson, R. Robinson and E. Schlittler, *Chemistry and Industry*, 653 (1955), and references cited therein.

(11) (a) R. Goutarel, M. M. Janot, A. Le Hir, H. Corrodi and V. Prelog, *Helv. Chim. Acta*, **37**, 1805 (1954); (b) A. Stoll, A. Hofmann and R. Brunner, *ibid.*, **38**, 270 (1955).

(12) *Cf.* M. W. Klohs, M. D. Draper, F. Keller, W. Malesh and F. J. Petracek, THIS JOURNAL, **76**, 1332 (1954).

(13) For leading references, *cf.* N. Neuss, H. E. Boaz and J. W. Forbes, *ibid.*, **76**, 3234 (1954).

Ajmalidine is a much weaker base (pK'_a 6.3 (80% DMF) and 6.6 (60% DMF)) than ajmaline (pK'_a 8.1 and 8.4, respectively) and such a shift of 1.8 pK'_a units has been observed recently[14] in the dihydroindole series in going from an α-aminoketone to the corresponding α-aminoalcohol. These data suggest that ajmalidine contains both a dihydroindole and five-membered ring ketone moiety with a basic nitrogen located on the carbon atom adjacent to the carbonyl group. It is conceivable that ajmalidine is a precursor or an isomerization product of ajmaline and if an experimental connection between these two alkaloids could be established, it would have an important bearing on the structure of ajmaline. Further work on ajmalidine is contemplated as soon as additional supplies of this alkaloid become available.

The reported[7] hypotensive and sedative action of the alkaloidal extract of *R. sellowii* is obviously due to the presence of reserpine which could be isolated in poor yield. The only fraction with a typical sedative action in rabbits[15] was the weakly basic, reserpine-containing fraction; none of the other fractions or single alkaloids displayed this type of pharmacological activity.

Acknowledgment.—The work at Wayne University was supported by a research grant from the American Heart Association; Mrs. Dolores Phillips was responsible for all ultraviolet and infrared spectra. The investigation in the Lilly Laboratories was aided by Drs. H. Boaz and H. Rose (physical data), Miss M. Livezey (technical assistance) and Mr. G. M. Maciak and Miss G. Beckman (microanalyses).

Experimental[16]

Extraction of the Root-bark of *Rauwolfia sellowii*.—The dried and well-ground root-bark (1.0 kg.) of the plant, collected and identified by R. W. in the State of São Paulo, was extracted exhaustively with boiling methanol (two 3.5-l portions) for 5 days. The total alcoholic extract was concentrated to 300 ml. *in vacuo*. To this concentrated solution, water (600 ml.) and glacial acetic acid (100 ml.) were added and the turbid suspension was then repeatedly extracted with hexane (4 × 250 ml.) to remove colored tarry material. The acid–aqueous layer was then extracted with chloroform (four 300-ml. portions) to separate chloroform-soluble acetates. The chloroform solution was next washed with 5% ammonium hydroxide solution, then with water, dried over anhydrous sodium sulfate and on removal of the solvent *in vacuo* 59 g. of crude fraction (A) was obtained.

The acid solution remaining after the chloroform extraction was cooled and adjusted to *p*H 7. The resulting precipitate was extracted with chloroform (4 × 300 ml.) and the aqueous layer was left aside for further treatment. The chloroform layer was washed with water, dried over anhydrous sodium sulfate and on removing the solvent *in vacuo*, there was obtained 12 g. of crude fraction (B).

The aqueous layer from the above operation was adjusted to *p*H 11 and extracted with chloroform–ether (4:1). After washing with water, the solvent was removed *in vacuo* when 0.9 g. of gummy material was obtained. No strong base, however, could be isolated by subsequent chromatography of this fraction over neutral alumina (activity II–III).

The tars separated in each stage of this isolation procedure were not investigated.

Separation of the Weak Bases from Fraction A.—The benzene solution of a 5-g. aliquot of fraction A was subjected to chromatographic separation on 125 g. of neutral alumina (activity II–III) and eluted with benzene, benzene–ether, ether, ether–chloroform, chloroform and chloroform–methanol mixtures. Seventy-five fractions, each 300 to 700 ml. in volume, were collected.

Isolation of Py-tetrahydroalstonine.—Fraction 2 (benzene eluted) yielded a crude base (5 mg.) which on single crystallization from methanol gave white needles (2 mg.), m.p. 220–225° dec. Further quantities (51 mg.) of this base were isolated from the rest (54 g.) of fraction A in a large scale chromatogram. A part (21 mg.) on two recrystallizations from methanol yielded 7 mg. of the alkaloid,[13,16] m.p. 222–224° dec. before drying and 228–230° dec. after drying overnight *in vacuo* at 100°.

Anal. Calcd. for $C_{21}H_{24}N_2O_3$: C, 71.57; H, 6.86. Found: C, 71.59; H, 6.98.

Isolation of Aricin.—Eluates 3–11 (benzene–ether and ether) yielded 1.59 g. of a reddish-brown oil which was rechromatographed and then crystallized from methanol yielding 130 mg. of stout rods of aricin, m.p. 187–188°. In the subsequent chromatography of the large batch (54 g.) of fraction A, it was observed that by using only 540 g. of alumina, aricin easily could be eluted with benzene. The dried crude base (23 g.) obtained in the corresponding fraction, was crystallized from methanol and afforded 13.5 g. of aricin identical in all respects[16] with an authentic sample kindly furnished by Prof. M. M. Janot.[11a]

Isolation of Tetraphyllicine.[5b]—Small quantities of tetraphyllicine could be isolated from the ether–chloroform (4:6) eluates. Additional quantities (60 mg.) of the substance were subsequently isolated from the large-batch operation representing the total material (54 g.). On repeated recrystallizations from acetone containing a little methanol, the needles melted at 305° before drying and 315–316° after drying overnight *in vacuo* at 100° with decomposition and simultaneous sublimation. Identity with the sample isolated[5b] from *R. tetraphylla* was demonstrated in the usual manner.[16]

Isolation of Reserpine.—Fraction 20–23, eluted with ether–chloroform (3:7 and 1:9), yielded 15 mg. of crude reserpine. Rechromatography of this material and crystallization from methanol gave pure reserpine, m.p. 263–265° dec., which was identical in every respect[16] with an authentic sample.

Isolation of Ajmaline.—Fractions 28–63, eluted with chloroform and chloroform–methanol (containing up to 5% of methanol), afforded upon two crystallizations from methanol 1.0 g. of ajmaline, m.p. 156–157°.

Separation of the Alkaloids from Fraction B.—A 3.0-g. portion of fraction B was chromatographed on 100 g. of neutral alumina (activity II–III) in the manner outlined above for fraction A, 145 fractions (ranging from 250–400 ml. in volume) having been collected.

Isolation of Tetraphyllicine and Ajmaline.—Fractions 16–32 (benzene–chloroform (8:2)) yielded 0.19 g. of crude solid. This was combined with 0.31 g. of similar material obtained by chromatography of the remainder (9.0 g.) of fraction B and after further purification furnished 0.1 g. of tetraphyllicine. The over-all yield from both fractions (A and B) was 0.16 g. (0.016%).

Fractions 46–128 (benzene with varying quantities of chloroform, up to chloroform–methanol 95:5) yielded 1.23 g. of ajmaline. The total yield of this alkaloid was *ca.* 1.2% taking into account the additional amounts isolated in the large-scale chromatogram of fraction A.

Isolation of Ajmalicine and Ajmalidine.—The mother liquors from all the aricin and ajmaline fractions derived from fraction A were combined and twice chromatographed. After removing additional quantities of aricin in that manner, a total of 9 mg. of ajmalicine, m.p. 254–256° dec., could be crystallized from methanol and identified by direct comparison.[16]

Fractions of 74–85 (ether–chloroform 1:9) from the second chromatogram yielded 36 mg. of ajmalidine which was recrystallized several times from methanol to afford 14 mg. of prisms, m.p. 241–242°. In a second, independent isolation from 1 kg. of root bark, 22 mg. of crude ajmalidine

(14) E. C. Kornfeld, *et al.*, in preparation.

(15) The pharmacological assay in rabbits was carried out by Mr. E. E. Swanson and associates of the Lilly Research Laboratories.

(16) All melting points were determined on the Kofler block. All known alkaloids were identified by comparison of their X-ray powder patterns, ultraviolet and infrared (chloroform solution) spectra and paper-chromatographic mobility with those of authentic specimens. All yields are based on the weight of the root.

was obtained. For analysis, a sample was dried at 100° and 0.05 mm.; pK'_a 6.3 (80% DMF) and 6.6 (60% DMF); $\lambda_{max}^{CHCl_3}$ 5.77 μ; λ_{max}^{EtOH} 247 and 295 mμ, ϵ 9150 and 3020 as compared to λ_{max}^{EtOH} 247 and 295 mμ, ϵ 8730 and 3070 for ajmaline.

Anal. Calcd. for $C_{20}H_{24}N_2O_2$: C, 74.04; H, 7.46; N, 8.64; mol. wt., 324.4. Found: C, 73.76, 74.01; H, 7.64, 7.65; N, 8.22; mol. wt. (electrometric titration), 317 ± 20.

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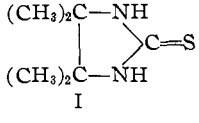
The Identity of Heilpern's "Pinacolylthiourea" and the Preparation of Authentic 2-Thiono-4,4,5,5-tetramethylimidazolidine

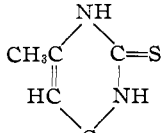
BY RALPH SAYRE

RECEIVED JULY 22, 1955

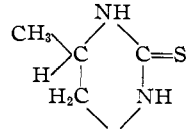
The reaction of aqueous ammonia with a mixture of acetone and carbon disulfide was investigated by Heilpern,[1] who isolated the principal product (his so-called "pinacolylthiourea") as crystals which melted with decomposition at 240–243°. As possible structures for the presumed new compound, to which he erroneously assigned the empirical formula $C_7H_{14}N_2S$, he considered the saturated cyclic thioureas I and II, deciding in favor of the former because permanganate oxidation of the thiourea gave 5,5-dimethylhydantoin (III) as the only product he could isolate and identify. The subsequent literature contains four brief and uninformative references[2] to the compound.



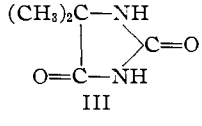
I



IV



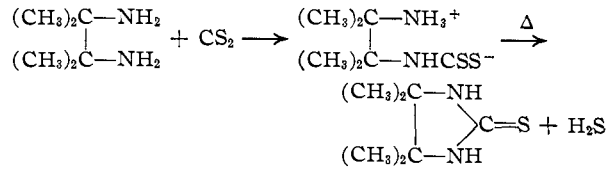
II



III

In 1946, repetition of Heilpern's preparation of the thiourea gave a product which, after six recrystallizations, melted with decomposition at 254–255°. Its infrared spectrum[3] showed fairly strong absorption at 1700 cm.$^{-1}$, interpreted by Dr. Fred Halverson of these laboratories as showing the presence of a C=C linkage and indicating that the thiourea was probably the tetrahydropyrimidine derivative IV. In verification of this conjecture, an almost identical curve was obtained in measuring the infrared absorption of an unsaturated cyclic thiourea $C_7H_{12}N_2S$, to which structure IV had been assigned by Traube[4] two years prior to Heilpern's publication. This compound, made by Traube's procedure from diacetonamine acid oxalate by reaction with potassium thiocyanate, was also prepared by various other published methods.[5]

Using the procedure by which Heilpern convinced himself of the structure of his "pinacolylthiourea," a pure specimen of 2-thiono-4,4,6-trimethyl-1,2,3,4-tetrahydropyrimidine, prepared by the method of Mathes[5b] and melting with decomposition at 254–255°, was subjected to permanganate oxidation; ring contraction occurred, giving 5,5-dimethylhydantoin as the only crystalline product. Conversely, in order to show the fallacy of Heilpern's assumption that one of the *gem*-dimethyl groups of the postulated symmetrical imidazolidine derivative I undergoes complete oxidation while the other remains intact, authentic 2-thiono-4,4,5,5-tetramethylimidazolidine was made by treating 2,3-dimethylbutane-2,3-diamine with carbon disulfide:



Its infrared spectrum showed no absorption band at 1700 cm.$^{-1}$. Permanganate oxidation of the new compound left both *gem*-dimethyl groups intact, giving a high yield of 4,4,5,5-tetramethyl-2-imidazolidone, which was also prepared by phosgenation of 2,3-dimethylbutane-2,3-diamine. The infrared spectrum of the imidazolidone showed extremely strong absorption at 1700 cm.$^{-1}$, attributable to the carbonyl group.

Experimental[6]

Permanganate Oxidation of IV.—To a well-stirred suspension of 4.69 g. (0.03 mole) of authentic 2-thiono-4,4,6-trimethyl-1,2,3,4-tetrahydropyrimidine in 100 ml. of water, 100-ml. portions of 0.125 *M* potassium permanganate were added at intervals ranging from 10 to 30 minutes, depending on the rate of consumption. After 1200 ml. had been added, it became necessary to heat the mixture and to add the permanganate in smaller portions. A total of 1740 ml. was required to complete the oxidation. The precipitated manganese dioxide was filtered off, and the strongly alkaline filtrate was slightly acidified with sulfuric acid, concentrated by vacuum distillation at about 40°, and finally evaporated

(1) J. Heilpern, *Monatsh. Chem.*, **17**, 229–244 (1896).

(2) W. Herzog, *Oesterr. Chem.-Ztg.*, **24**, 76 (1921); P. C. Ray and R. Das, *J. Chem. Soc.*, **121**, 326 (1922); F. G. Moses, R. W. Hess and R. L. Perkins, U. S. Patent 1,801,319 (1931); W. G. Bywater, D. A. McGinty and N. D. Jenesel, *J. Pharmacol. Expl. Therap.*, **85**, 14 (1945). The thiourea is listed in Beilstein (4th Ed.) as a thioimidazolidone (Vol. XXIV, 12); and a poorly characterized derivative which Heilpern obtained is listed as an imidazoline (Vol. XXIII, 351).

(3) The infrared curves referred to in this paper were obtained in these laboratories under the direction of Dr. R. C. Gore and have been deposited as Document number 4687 with the ADI Auxiliary Publications Project, Photoduplication Service, Library of Congress, Washington 25, D. C. A copy may be secured by citing the Document number and by remitting in advance $1.25 for photoprints, or $1.25 for 35 mm. microfilm payable to: Chief, Photoduplication Service, Library of Congress.

(4) W. Traube, *Ber.*, **27**, 277 (1894).

(5) (a) W. P. ter Horst, U. S. Patents 2,131,790 (1938) and 2,234,848 (1941); (b) R. A. Mathes, F. D. Stewart and F. Swedish, Jr., THIS JOURNAL, **70**, 1452 (1948); (c) K. C. Roberts and R. J. Moualim, British Patent 654,609 (1951). Without adducing any evidence that the compound possesses mercaptan-like properties, these chemists have generally formulated it as a 2-mercapto-1,4(or 3,4)-dihydropyrimidine derivative. The present work, besides confirming Traube's statement that it is almost insoluble in aqueous alkali, showed that it does not react with iodine to give a disulfide.

(6) Melting points by capillary method, corrected for stem emergence.