1.4 ml. (0.01 mole) of triethylamine, the mixture allowed to stand for 45 minutes at 25°, the clear solution washed into a distilling flask with 250 ml. of water containing 15 ml. of 2 *N* sulfuric acid, the contents of the flask steam distilled, the distillate (50 ml.) extracted with ether, the ethereal extract dried over magnesium sulfate, the solvent removed and the oily residue allowed to react with dinitrochlorobenzene in the presence of alkali[42] to give 0.36 g. (68%) of 2,4-dinitrodiphenyl sulfide, m.p. 118–119°. The residue remaining after the steam distillation was filtered, the solid washed with water, dried, and recrystallized from ethanol to give 0.52 g. (98%) of *sym*-diphenylurea, m.p. 233–234°.

Attempted Reaction of N,S-Diphenylthiocarbamate with Isopropyl Alcohol and with Ethanol.—To a solution of 0.57 g. (0.0025 mole) of N,S-diphenylthiocarbamate in 0.5 ml. of dry isopropyl alcohol and 10 ml. of anhydrous dioxane was added 0.5 ml. of triethylamine, the reaction mixture allowed to stand at 25° for 45 minutes, then poured into 50 ml. of ice water, the precipitate recovered and dried to give 0.48 g. of the original amide, m.p. 124–125°. Practically the same result was obtained when the solution of isopropyl alcohol and dioxane was replaced by absolute ethanol.

Attempted Reaction of N-Phenyl-N-methyl-S-phenylthiocarbamate with Several Primary and Secondary Amines.—A solution of 0.61 g. of N-phenyl-N-methyl-S-phenylthiocarbamate in 15 ml. of diethylamine was allowed to stand at 25° for 3 hours, the solution then heated under refluxing conditions for 2 hours, cooled, and the solvent removed in a stream of illuminating gas to give 0.61 g. of the original amide, m.p. 69–70°. Essentially the same results were obtained when the amide was treated with a toluene solution of methylamine at 25°, a toluene solution of aniline and triethylamine at 25°, and a dioxane solution of N-methylaniline and triethylamine at the temperature of the refluxing reaction mixture.

Attempted Reaction of N-Pentamethylene-S-phenylthiocarbamate with Diethylamine.—To a solution of 2.21 g. (0.01 mole) of N-pentamethylene-S-phenylthiocarbamate in 15 ml. of dry toluene was added 5.1 ml. (0.05 mole) of diethylamine, the solution allowed to stand overnight and then heated under refluxing conditions for 2 hours. The solvent was evaporated in a stream of illuminating gas, the oily residue triturated with 20 ml. of 2 *N* hydrochloric acid, the solid collected, washed with water and air-dried to give 2.22 g. of product, m.p. 60–62°. Recrystallization of this material from 60–80° ligroin gave 1.59 g. (72%) of the original amide, m.p. 59–60°.

(42) N. D. Cheronis and J. B. Entrikin, "Semimicro Qualitative Organic Analysis," T. Y. Crowell Co., New York, N. Y., 1947.

Attempted Reaction of N-Phenylurethan with Aniline.—To a solution of 0.83 g. of N-phenylurethan in 10 ml. of anhydrous dioxane was added 1.0 ml. of aniline and 1.4 ml. of triethylamine, the reaction mixture allowed to stand at 25° for 5 hours, poured into 50 ml. of ice-water, the crystalline solid collected and dried to give 0.80 g. of the original urethan, m.p. 48–49°.

Reaction of Phenylthiocarbonyl Chloride with DL-Phenylalanine.—S-Phenylthiocarbonyl chloride, 12.95 g. (0.075 mole), was weighed into a small separatory funnel and the acid chloride was added in small portions to a vigorously stirred solution of 4.13 g. (0.025 mole) of DL-phenylalanine in 15 ml. of 3 *N* aqueous sodium hydroxide and 10 ml. of water. When *ca.* one-fifth of the acid chloride had been added, an additional 10 ml. of the aqueous alkali was introduced into the reaction mixture followed by more of the acid chloride until *ca.* 4 g. of the latter remained in the funnel. Since no reaction was apparent at this stage, stirring was discontinued and the reaction mixture allowed to stand overnight. After an interval of 9 hours, during which time a precipitate had separated, an additional 15 ml. of 3 *N* alkali was added followed by the remainder of the acid chloride which was added dropwise and with stirring. After all of the acid chloride had been added, a final portion of 10 ml. of 3 *N* alkali was added and the reaction mixture stirred for an additional 8 hours. The solid was then collected, washed with water and dried to give 9.20 g. (99.5%) of diphenyl dithiocarbonate, m.p. 43.5–44°. The alkaline filtrate was quantitatively transferred to a flask equipped with a dropping funnel and an absorption train for the collection of carbon dioxide. The system was swept free of air by a stream of nitrogen, the carbon dioxide absorption tube weighed, the solution carefully acidified with 2 *N* sulfuric acid to *p*H 5 and the evolved carbon dioxide swept into the absorption tube by a stream of nitrogen. The amount of carbon dioxide collected, corrected for the amount of carbonate present in the added alkali and that remaining in solution at 20° was 1.11 g. or 0.025 mole. The solid which had precipitated from the alkaline solution upon acidification was collected, washed with water and dried to give 4.27 g. (96%) of N,N′-carbonylbis-DL-phenylalanine. Thus from the reaction of 2 moles of the α-amino acid with 6 moles of the acid chloride in the presence of an excess of aqueous sodium hydroxide there was obtained 1 mole of the urea, 3 moles of diphenyldithiocarbonate and 2 moles of carbon dioxide.

The authors wish to express their indebtedness to Mr. T. H. Applewhite for his assistance in the course of this investigation.

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[JOINT CONTRIBUTION FROM THE SAMUEL C. HOOKER LABORATORY OF THE DEPARTMENT OF CHEMISTRY OF WAYNE UNIVERSITY AND THE INSTITUTO DE QUIMICA DE LA UNIVERSIDAD NACIONAL AUTONOMA DE MEXICO]

Alkaloid Studies. IV.[1] The Isolation of Reserpine, Serpentine and Ajmaline from *Rauwolfia heterophylla* Roem. and Schult.[2]

BY CARL DJERASSI, MARVIN GORMAN,[3] A. L. NUSSBAUM[4] AND JESUS REYNOSO

RECEIVED APRIL 6, 1954

The alkaloids reserpine, serpentine and ajmaline have been isolated from the Mexican and Guatemalan *Rauwolfia heterophylla*. The presence of γ-sitosterol and sucrose also has been noted.

Alkaloids have been encountered in a number of Asian and African *Rauwolfia* species,[5,6] but the majority of the work has centered on *Rauwolfia serpentina* Benth. which has been employed in India for the treatment of hypertension and other clinical conditions.[5–7] Enormous interest in the Indian *R. serpentina* was created recently by the isolation by

(1) Paper III, C. Djerassi, C. R. Smith, S. P. Marfey, R. N. McDonald, A. J. Lemin, S. K. Figdor and H. Estrada, THIS JOURNAL, **76**, 3215 (1954).

(2) We are grateful to the Rockefeller Foundation for funds in support of the plant collections.

(3) Pfizer Predoctorate Research Fellow, 1953–1954.

(4) U. S. Public Health Service Predoctorate Research Fellow, 1952–1954.

(5) T. A. Henry, "The Plant Alkaloids," Blakiston Co., Philadelphia, Penna., 1949, pp. 761–765; L. Marion in R. H. F. Manske and H. L. Holmes, "The Alkaloids," Academic Press, Inc., New York, N. Y., 1952, Vol. II, pp. 424–429.

(6) A. Chatterjee in L. Zechmeister, "Progress in the Chemistry of Organic Natural Products," Springer, Vienna, 1953, Vol. X.

(7) *Inter al.*, M. D. Chakravarti, *Brit. Med. J.*, 1390 (1953).

Schlittler, *et al.*,[8] of a weakly basic alkaloid, reserpine, which was shown[9] to be the active hypotensive and sedative principle and which is now being employed extensively in clinical practice in this country.[10]

Of the *Rauwolfia* species indigenous to the American continent, *R. heterophylla* Roem. and Schult. is by far the most abundant since it grows widely in Mexico,[11] Guatemala ("chalchupa"),[12] Costa Rica,[13] and Colombia ("pinique-pinique").[14] In view of the extensive work now being carried out in our laboratories on natural products from Latin American plant sources, it appeared of interest to investigate *Rauwolfia heterophylla*, particularly since earlier reports[12,15,16] indicated the presence of alkaloids and recorded the pharmacological effects of crude extracts.[12,15–17] The earlier chemical studies[12,15] were limited to the isolation of two amorphous products, named "chalchupin A" and "chalchupin B" and to which were assigned the unlikely formulas $C_{14}H_{21}N_3O_{12}$ and $C_{15}H_{24}N_6O_{11}$.

The plant material employed in the present investigation was in part collected by one of us near Tomellin, Oaxaca, in Mexico and identified botanically by Prof. M. Martinez,[11] while the Guatemalan specimens were obtained through the courtesy of Mr. Mario Wunderlich of Guatemala City. At the time that the present investigation was initiated, no details were available on the isolation procedure of reserpine from *R. serpentina*[18] nor was the empirical formula of this alkaloid known.[19] Our first attempts, based on the presumptive lability of reserpine, involved direct chromatography of the benzene-soluble portion of the methanolic plant extract and resulted in the isolation of crystalline reserpine and *l*-narcotine.[20] *R. heterophylla* is thus the second *Rauwolfia* species in which the presence of the widely sought-after reserpine has been recorded.[21] Subsequent experiments have led to an improved isolation scheme which is outlined in the Experimental part of this paper.

Having established the presence of this alkaloid in *R. heterophylla*, it appeared of interest to determine whether any additional alkaloids, encountered in other *Rauwolfia* species, could be isolated. Indeed, when a crude alkaloid fraction was subjected to a countercurrent distribution at *p*H 6.6, crystalline ajmaline[22,23] and serpentine[22–25] could be obtained. It appears, therefore, that insofar as its main alkaloidal components are concerned, the Latin American *R. heterophylla* and the Asian *R. serpentina* are qualitatively quite similar.

Acknowledgment.—We are greatly indebted to Messrs. Mario and Edgar Wunderlich of Guatemala City for assistance in securing plant material, to Dr. M. W. Klohs of Riker Laboratories, Los Angeles, Calif., and Dr. Oskar Wintersteiner of the Squibb Institute for Medical Research, New Brunswick, N. J., for samples of authentic *Rauwolfia* alkaloids. We are also grateful to Prof. M. Martinez, Instituto de Biologia de la Universidad Nacional Autonoma de Mexico, for assistance in the botanical identification.

Experimental[26]

Cold Extraction. Isolation of Reserpine and γ-Sitosterol.—Whole roots of *Rauwolfia heterophylla* from either Mexico or Guatemala were cut into pieces *ca.* 2 in. long, dried to constant weight in a steam oven at 85° and then ground in a Wiley mill to pass a 1-mm. screen. A portion (250 g.) of this ground root was shaken in an atmosphere of nitrogen

(8) J. M. Müller, E. Schlittler and H. J. Bein, *Experientia*, **8**, 338 (1952).

(9) H. J. Bein, *ibid.*, **9**, 107 (1953).

(10) *Cf.* "Conference on Reserpine and Other Alkaloids of *Rauwolfia serpentina:* Chemistry, Pharmacology and Clinical Applications," *Ann. N. Y. Acad. Scien.*, **59**, 1 (1954).

(11) M. Martinez, "Las Plantas Medicinales de Mexico," Ediciones Botas, Mexico, D.F., 1944, p. 356.

(12) E. C. Deger, *Arch. Pharm.*, **275**, 496 (1937).

(13) Private communication from Dr. J. Leon, Turrialba, Costa Rica.

(14) M. M. Janot and R. Mendoza (*Compt. rend.*, **209**, 653 (1939)) carried out a direct botanical comparison between the Colombian and Guatemalan (ref. 12) plant specimens.

(15) R. Paris and R. Mendoza D., *Bull. Sci. Pharmacol.*, **48**, 146 (1941).

(16) I. Ochoterena, *Anal. Inst. Biol. Mex.*, **9**, 85 (1938).

(17) Raymond-Hamet, *Compt. rend.*, **209**, 384, 599 (1939).

(18) The details of this isolation scheme—quite different from that employed by us—and the significant degradation experiments on reserpine have now been published by L. Dorfman, A. Furlenmeier, C. F. Huebner, R. Lucas, H. B. MacPhillamy, J. M. Müller, E. Schlittler and A. F. St. André, *Helv. Chim. Acta*, **37**, 59 (1954).

(19) The correct empirical formula $C_{33}H_{40}N_2O_9$, at which we arrived independently (ref. 20), has since been published by A. Furlenmeier, R. Lucas, H. B. MacPhillamy, J. M. Müller and E. Schlittler, *Experientia*, **9**, 331 (1953), and by N. Neuss, H. E. Boaz and J. W. Forbes, THIS JOURNAL, **75**, 4870 (1953). *Cf.* M. W. Klohs, M. D. Draper, F. Keller and F. J. Petracek, *ibid.*, **75**, 4867 (1953).

(20) C. Djerassi, M. Gorman, A. L. Nussbaum and J. Reynoso, *ibid.*, **75**, 5446 (1953). The isolation of the opium alkaloid narcotine reported in our communication was surprising since all of the *Rauwolfia* alkaloids hitherto encountered[5,6] possess an indole skeleton. Subsequently, it was discovered that while reserpine was obtained in each instance, narcotine was not encountered in certain batches which were processed up to the methanolic residue stage at the University of Mexico. A detailed experimental check revealed that the only plant material from which narcotine could be obtained was one in which the fresh plants had been shipped from Guatemala to Detroit and the roots milled by a *commercial* laboratory; we found subsequently that this mill had been used shortly before for the grinding of opium. When a new batch of Guatemalan plant specimens was processed completely in our laboratory at Wayne University, no narcotine was encountered and none was ever isolated from Mexican sources. It would appear, therefore, that the earlier reported isolation of narcotine was due to contamination. However, it should be noted that A. Hofmann (*Helv. Chim. Acta*, **37**, 849 (1954)) has recently reported the isolation of two opium alkaloids, thebaine and papaverine, from the Indian *Rauwolfia serpentina*.

(21) Since submission of this manuscript there appeared reports by M. W. Klohs, M. D. Draper, F. Keller and F. J. Petracek (THIS JOURNAL, **76**, 1381 (1954)) and by J. Poisson, A. Le Hir, R. Goutarel and M. M. Janot (*Compt. rend.*, **238**, 1607 (1954)) describing the isolation of reserpine from *R. canescens* Linn., and *R. vomitoria*, respectively.

(22) First isolated from *R. serpentina* by S. S. Siddiqui and R. H. Siddiqui, *J. Ind. Chem. Soc.*, **8**, 667 (1931); **9**, 539 (1932); **12**, 37 (1935); and by L. Van Itallie and A. J. Steenhauer, *Arch. Pharm.*, **270**, 313 (1932).

(23) For structure studies, *cf.* D. Mukherji, R. Robinson and E. Schlittler, *Experientia*, **5**, 215 (1949), and A. Chatterjee and S. Bose, *ibid.*, **9**, 254 (1953).

(24) Since completion of this manuscript there appeared an article by M. M. Janot, R. Goutarel and A. Le Hir (*Compt. rend.*, **238**, 720 (1954)) in which is recorded the isolation of serpentine from *R. heterophylla*.

(25) For structure studies, *cf.* E. Schlittler and H. Schwarz, *Helv. Chim. Acta*, **33**, 1463 (1950); F. Bader and H. Schwarz, *ibid.*, **35**, 1594 (1952); M. W. Klohs, M. D. Draper, F. Keller, W. Malesh and F. J. Petracek, THIS JOURNAL, **76**, 1332 (1954); F. L. Weisenborn, M. Moore and P. A. Diassi, *Chemistry and Industry*, 375 (1954).

(26) All melting points are uncorrected. Rotations were measured in chloroform and ultraviolet absorption spectra in absolute ethanol solution. The infrared spectra were obtained with a Baird Associates double beam recording infrared spectrophotometer employing a cell thickness to 0.1 mm. We are indebted to Miss Phylis Tocco (Wayne University) and Mr. Joseph F. Alicino (Metuchen, N. J.), for the microanalyses.

for 15 minutes with 2.5 l. of the following solution (A)[27]: 1.7 l. of ether, 550 cc. of benzene and 170 cc. of 90% ethanol. Aqueous ammonium hydroxide (1:1, 425 cc.) was then added and shaking was continued for 48 hours. The extract was decanted from the ground root, the latter was washed three times with 100-cc. portions of solution A and the shaking process was repeated twice with 1 l. each of solution A. The combined extracts and washings were dried over sodium sulfate and evaporated to dryness *in vacuo*.[28] The residue, dissolved in 200 cc. of benzene, was chromatographed on 75 g. of neutral alumina (activity II). The ether eluates, after crystallization from acetone, yielded 85 mg. of *γ-sitosterol*,[29] m.p. 147–148°, $[\alpha]^{25}D$ −46°; acetate, m.p. 139–140°, $[\alpha]^{25}D$ −46°; benzoate, m.p. 147–149°, $[\alpha]^{25}D$ −19°.

Anal. Calcd. for $C_{36}H_{54}O_2$: C, 83.34; H, 10.49. Found: C, 83.60; H, 10.35.

The pooled ether–chloroform eluates after crystallization from methanol yielded 117 mg. of **reserpine** with m.p. 262–263°, undepressed upon admixture with an authentic sample isolated from *R. serpentina* and kindly supplied by Dr. M. W. Klohs, $[\alpha]^{22}D$ −115°, λ_{max}^{EtOH} 268 mμ (log ε 4.15) and shoulder at 288–297 mμ (log ε 3.95), infrared spectrum[20] identical with that of an authentic specimen.

Anal. Calcd. for $C_{27}H_{22}N_2O_3(OCH_3)_6$: C, 65.11; H, 6.62; N, 4.60; methoxyl, 30.59; mol. wt., 608. Found: C, 65.25; H, 6.42; N, 4.54; methoxyl, 29.83; mol. wt. (Rast), 619.

Hot Extraction. Isolation of Serpentine, Ajmaline and Sucrose.—The ground root (250 g.) was extracted continuously for 60 hours in an atmosphere of nitrogen in a Soxhlet apparatus with 2.5 l. of absolute methanol. Upon concentration of the extract to a volume of 150 cc., colorless crystals appeared which were filtered and washed with chloroform furnishing 0.43 g. of **sucrose**, m.p. 186°, identified by mixture melting point and infrared comparison. The filtrate was diluted with 300 cc. of amyl alcohol and concentration (at 18 mm.) was continued until no more methanol remained. The resulting suspension was filtered, the resinous precipitate having been discarded, and then concentrated to a thick sirup. This residue was leached several times with 5% hydrochloric acid, the acid extracts were made basic at 0° with ammonium hydroxide[30] and extracted exhaustively with chloroform. After drying and evaporation of the solvent, the residue (2.4 g.) was subjected to a ten-stage countercurrent distribution between 100 cc. each of chloroform and citrate-phosphate buffer (*p*H 6.6) with the following results:

Fraction	Wt., g.	Fraction	Wt., g.
0	0.65	6	0.05
1	.22	7	.035
2	.17	8	.09
3	.13	9	.205
4	.09	10	.63
5	.08		

The amorphous material in fractions 0 and 1 was triturated with 50 cc. of 10% acetic acid, filtered, the filtrate was made basic with ammonium hydroxide and again filtered. The ammoniacal solution was made strongly alkaline at 0° with 20% sodium hydroxide, the resulting precipitate was collected, taken up in chloroform, dried and the solvent was evaporated. Crystallization of the residue from absolute ethanol furnished 0.13 g. of bright yellow crystals of **serpentine**[23,25] with m.p. 156–157°; identity was established by mixture melting point and infrared comparison with an authentic specimen furnished by Dr. M. W. Klohs.

Trituration of fractions 2–5 with methanol resulted in slow crystallization and recrystallization of the solid from methanol yielded 0.053 g. of colorless crystals of **ajmaline**, m.p. 158–160°, identical in all respects (mixture m.p. and infrared spectrum) with an authentic sample.

(27) This is a modification of the solvent system given in the British Pharmaceutical Codex, Pharmaceutical Press, London, 1949, pp. 762–763.

(28) After removal of the solvent, a colorless solid was observed to sublime from the residual material. This proved to be acetamide, presumably an artifact of the isolation procedure.

(29) Reported (Elsevier's "Encyclopedia of Organic Chemistry," **14**, 91 (1940)): γ-sitosterol, m.p. 147–148°, $[\alpha]D$ −43°; acetate, m.p. 143–144°, $[\alpha]D$ −45.3°; benzoate, m.p. 152°, $[\alpha]D$ −19.6°.

(30) This treatment is not sufficient to liberate all of the serpentine,

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NOTES

γ,γ-Bis-(*p*-hydroxyphenyl)-valeric Acid

BY ALFRED R. BADER AND ANTHONY D. KONTOWICZ

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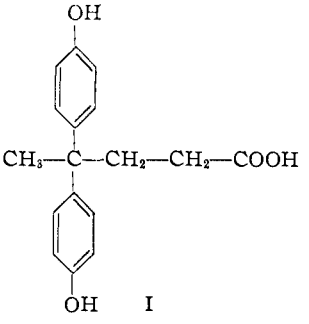
A study of the reaction of phenol with levulinic acid has shown that the condensation to yield the bisphenol I proceeds easily in the presence of acids such as sulfuric, hydrochloric and phosphoric; with polyphosphoric acid a mixture of condensation products and phenyl levulinate[1] results.



I

The bisphenol I is dimorphic; an amorphous modification forms crystalline solvates with aromatic hydrocarbons, and a crystalline, solvent-free modification melts at 171–172°.

Experimental

γ,γ-Bis-(*p*-hydroxyphenyl)-valeric Acid (I).—A cooled mixture of 94 g. (1 mole) of phenol, 58 g. (0.5 mole) of levulinic acid, 45 g. of water and 180 g. of concd. sulfuric acid was stirred at 25° for 20 hours. The reaction is slightly exothermic. The mixture was diluted with water and extracted with ethyl acetate. The organic solution was in turn extracted exhaustively with aqueous sodium bicarbonate, stripped and distilled to yield 20 g. of unreacted phenol. The almost colorless bicarbonate extract was acidified, extracted with ether and the washed ether extract stripped *in vacuo* to yield 87 g. (0.30 mole, 77% yield based on unrecovered phenol) of I, an almost colorless glass, m.p. *ca.* 90°, acid value found 192, calcd. 195.

(1) A. R. Bader and A. D. Kontowicz, THIS JOURNAL, **75**, 5416 (1953).