ent positions absorb closely to each other in this region. One distinction could be made: 3α,5α-structures absorbed near 1018 (both solid and solution) while the other spatial isomers gave rise to bands at higher frequencies. The 3β,5α-isomer absorbed near 1033 (F) or 1026 (S), the 3α,5β-structures near 1023 (F) or 1029 (S) and the 3β,5β-form near 1028 (F) or 1022 cm.$^{-1}$ (S). It is of interest to note that solid film frequencies of the 3α,5β-form were lower than those of solutions.

The spectra of free and acetylated compounds also were scrutinized for a combination of frequencies which might be associated with steroid structure. This difficult problem of being unequivocally able to establish that a substance is a steroid on the basis of infrared spectrometry remains unsolved. It is empirically known that many bands of varying intensities occur in steroid spectra and the present investigation also was concerned with locating a combination of frequencies which eventually may be an identifying mark for steroid compounds. It was found that weak to medium weak bands occurred near 1311, 1265, 1242, 1217 and 1127 cm.$^{-1}$ in all the spectra of the free steroids. A medium weak absorption near 900 cm.$^{-1}$ appeared in the curves of free 21-hydroxy, 21-desoxy and C-27 compounds but was only present in approximately 75% of C-19 spectra. Furthermore the latter contained a band near 791 which was absent in the other curves while the steroids with more than 19 carbons gave rise to an absorption of weak intensity near 885 cm.$^{-1}$.

Except for the 1311 cm.$^{-1}$ band the relationship seemed to apply to the acetylated derivatives. Naturally the 1266 and 1242 cm.$^{-1}$ bands were obliterated by the acetate group absorption. An interesting observation was that a significant intensification of the 1157 cm.$^{-1}$ band occurred in the spectra of 21-desoxy steroids not having a 17-hydroxyl group (allopregnan-3α-ol-20-one, pregnan-3α-ol-11,20-dione, pregnan-3α-ol-20-one and allopregnan-3β-ol-20-one). It remains to be seen whether the distinctions between C-19 and C-21 molecules and the seemingly characteristic frequencies of tetrahydro steroids will apply to other groups of steroid compounds. It also must be ascertained whether non-steroid spectra will interfere with steroid assignment on the basis of a particular combination of frequencies.

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[CONTRIBUTION FROM RIKER LABORATORIES]

Alkaloids of *Rauwolfia serpentina* Benth. V.[1] Rescinnamine

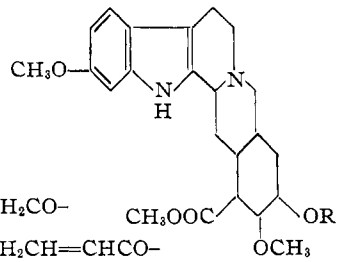
BY M. W. KLOHS, M. D. DRAPER AND F. KELLER

RECEIVED NOVEMBER 8, 1954

The isolation and characterization of rescinnamine, a new alkaloid from *Rauwolfia serpentina* Benth possessing pronounced hypotensive and sedative activity, is reported. Rescinnamine ($C_{35}H_{42}O_9N_2$) has been shown to be the 3,4,5-trimethoxycinnamic acid ester of methyl reserpate.

The Indian plant *Rauwolfia serpentina* Benth has aroused widespread interest because of its therapeutic value as a hypotensive and sedative agent, and has been the subject of numerous chemical investigations[2] in a search for the components responsible for this physiological activity. The isolation of one of these, reserpine, has been reported recently by Mueller, *et al.*,[3] and independently by this and other laboratories.[4-6]

Extensive pharmacological[7] and clinical[7] comparison of reserpine and an alkaloidal extract[8] of *Rauwolfia serpentina* indicated, however, that the extract possessed a greater degree of activity than could be accounted for by its reserpine content, thus suggesting the presence of other potent alkaloids which our initial chemical investigation had not revealed. On comparing reserpine (I)[9] with the inactive alkaloids present in this species, a



I, R = 3,4,5(OCH_3)$_3$$C_6H_2CO$–
II, R = H
III, R = 3,4,5(OCH_3)$_3$$C_6H_2CH$=$CHCO$–

significant structural difference relating to its biological activity is manifested by its ester character wherein an aromatic acid is conjugated with an alkaloidal alcohol. The importance of this grouping in potentiating biological activity in this series is shown by the relative inactivity of methyl reserpate (II) when compared with its conjugate

(1) A preliminary report of this investigation appeared in a previous communication; *cf.* M. W. Klohs, M. D. Draper and F. Keller, THIS JOURNAL, **76**, 2843 (1954).

(2) For a comprehensive review of earlier work see Asima Chatterjee in L. Zechmeister "Progress in the Chemistry of Organic Natural Products," Vol. 10, Springer-Verlag, Vienna, Austria, 1953, pp. 390-417. For a summary of more recent work see E. Schlittler, J. A. Schneider and A. J. Plummer, *Angew. Chem.*, **66**, 386 (1954).

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

(4) M. W. Klohs, M. D. Draper, F. Keller and F. J. Petracek, THIS JOURNAL, **75**, 4867 (1953).

(5) N. Neuss, H. E. Boaz and J. W. Forbes, *ibid.*, **75**, 4870 (1953).

(6) C. Djerassi, M. Gorman, A. L. Nussbaum and J. Reynoso, *ibid.*, **75**, 5446 (1953).

(7) This work was carried out by the Biological Sciences and Clinical sections of this Laboratory.

(8) This investigation was carried out on an alkaloidal extract of *Rauwolfia serpentina*, generically designated "alseroxylon," which is available from Riker Laboratories, Inc., Los Angeles, Calif.

(9) L. Dorfman, A. Furlenmeier, C. F. Huebner, R. Lucas, H. B. MacPhillamy, J. M. Mueller, E. Schlittler, R. Schwyzer and A. F. St. Andre, *Helv. Chim. Acta*, **37**, 59 (1954).

reserpine. The possibility that other similarly constituted active alkaloids were present prompted a study of the non-volatile acid fraction resulting from the hydrolysis of the original extract as a simple means of justifying this assumption.

By employing the technique of countercurrent distribution, it was possible to separate this material into two major components, which were subsequently identified as 3,4,5-trimethoxybenzoic acid and 3,4,5-trimethoxycinnamic acid; while the presence of the former acid was expected due to the reserpine content of the extract, the isolation of the latter acid pointed to the presence of a hitherto undescribed ester alkaloid. It was found further, by using this method for following the course of the ester through the isolation pattern, that like reserpine, the bulk of the new conjugate could be concentrated in the benzene-soluble portion of the total alkaloids.

On subjecting this concentrate to chromatographic separation on acid-washed alumina, a fraction was obtained which had an infrared spectrum compatible with that of an ester of 3,4,5-trimethoxycinnamic acid; in addition the ultraviolet absorption spectrum was observed to be identical with that of a curve obtained by a summation of the extinction coefficients of methyl reserpate and methyl 3,4,5-trimethoxycinnamate. The fraction readily crystallized from benzene affording a new alkaloid, **rescinnamine,**[10] which indeed yielded 3,4,5-trimethoxycinnamic acid and reserpic acid on basic hydrolysis. A comparison of rescinnamine with the 3,4,5-trimethoxycinnamic acid ester of methyl reserpate (III), prepared in connection with a separate project, showed them to be identical.

Pharmacology.—Pharmacological studies on rescinnamine show it to have physiological activity similar to reserpine.[11,12]

Experimental

Identification of the Non-volatile Acid Constituents Resulting from the Hydrolysis of an Alkaloidal Extract of *Rauwolfia serpentina*.—The alkaloidal extract[8] (50 g.) of *Rauwolfia serpentina* was refluxed for 2 hours in 1 *N* methanolic sodium hydroxide (1 l.); at the end of this time, the solution was concentrated *in vacuo* on the steam-bath to 200 ml., diluted with water (400 ml.), adjusted to *p*H 2 with concentrated hydrochloric acid, and extracted four times with 100-ml. portions of ether. The combined ether extracts were dried over anhydrous sodium sulfate and then concentrated to dryness *in vacuo*.

The acidic fraction obtained above (2.9 g.) then was subjected to a 24-plate countercurrent distribution using 1 *M* acetate buffer *p*H 5.5 and chloroform as the solvent system (50 ml. each phase). The optical densities of the aqueous phases from each tube were read at 260 mμ and the results when plotted yielded a well-defined band with a peak at tube 9 corresponding to the distribution coefficient observed for 3,4,5-trimethoxybenzoic acid. There was also evidence of substantial quantities of material in tubes 0–1. The acids in the two peak areas were recovered by acidifying the contents of the tubes with concentrated hydrochloric acid and extracting with chloroform. The material recovered from the area of the peak at tube 9 was crystallized from water yielding 3,4,5-trimethoxybenzoic acid, m.p. 167–168°; identity was established by mixture melting point and comparison of infrared and ultraviolet absorption spectra with an authentic sample.

The material recovered from tubes 0–1 was crystallized by dissolving in a minimum of methanol, diluting with an excess of Skellysolve A and concentrating on the steam-bath, yielding white needles, m.p. 125–126°. The acid was identified as 3,4,5-trimethoxycinnamic acid by comparison with an authentic sample.

Preliminary Fractionation of the Alkaloidal Extract of *Rauwolfia serpentina*.—The alkaloidal extract[8,13] (100 g.) was dissolved in methanol (170 ml.) and benzene (3500 ml.) was added with stirring. The stirring was continued for one hour and at the end of this time, the solution was filtered clear of the amorphous precipitate. The procedure was repeated on this precipitate and the combined benzene extracts were concentrated *in vacuo* on the steam-bath to 4800 ml. The solution was allowed to stand at room temperature for one hour and a bright yellow powder (25 g.) which had settled out was removed by filtration. The benzene solution was then taken to dryness *in vacuo* yielding a tan resin (15 g.). This material was dissolved in 2.5% acetic acid in methanol (150 ml.) and ammonium hydroxide was added to *p*H 8.5. On standing, crystalline reserpine (5.3 g.) was obtained. The mother liquors were taken to dryness *in vacuo*.

Isolation of Rescinnamine.—A portion of the above mother liquor fraction (5 g.) was dissolved in a minimum of benzene and chromatographed on 100 g. of Merck acid-washed alumina. The fractions eluted with chloroform through chloroform–1% methanol on crystallization from benzene yielded rescinnamine (0.7 g.). After several recrystallizations from acetone–water, rescinnamine melted at 238–239° (vac.), $[\alpha]^{24}D$ −97 ± 2 (*c* 1.0 in $CHCl_3$). A 24-plate countercurrent distribution in a Craig glass apparatus between 5% aqueous acetic acid and methylchloroform gave a single band which corresponded with a theoretical curve for a single substance (K = 1.18). The infrared spectrum (Nujol) showed well defined bands at 2.95 μ (–NH), 5.80 μ and 5.90 μ (ester carbonyl), 6.19 μ (—C=C—; 6-methoxyindole)[13a] (—C=C—) and 6.3 μ, 6.7 μ (aromatic). The ultraviolet spectrum showed $\lambda_{max}^{alc.}$ (log ε) 229 mμ (4.73), 302 mμ (4.39); $\lambda_{min}^{alc.}$ (log ε) 258 mμ (3.88). For analysis the sample was dried to constant weight at 100° (2 mm.).[14]

Anal. Calcd. for $C_{35}H_{42}O_9N_2$: C, 66.23; H, 6.67; N, 4.41; OCH_3, 29.34; mol. wt., 634.71. Found: C, 66.24; H, 6.62; N, 4.45; OCH_3, 28.81; equiv. wt., 636[15]; pK_a', 6.4.

Hydrolytic Cleavage of Rescinnamine to Reserpic Acid and 3,4,5-Trimethoxycinnamic Acid.—Rescinnamine (0.5 g.) was refluxed for 45 minutes in a solution of methanol (30 ml.)–water (10 ml.) to which 3 ml. of 12 *N* sodium hydroxide had been added. At the end of this time, the slightly yellow solution was concentrated *in vacuo* to remove the methanol. The solution was then diluted with an additional 20 ml. of water, cooled, adjusted to *p*H 2.0 by the addition of concd. hydrochloric acid, and extracted three times with 20-ml. portions of chloroform. The combined chloroform extracts were concentrated under vacuum to a resinous mass which on standing crystallized as white needles (135 mg.). On recrystallization the material melted at 126–127° and showed no depression upon admixture with an authentic sample of 3,4,5-trimethoxycinnamic acid. The infrared and ultraviolet spectra were identical with those of the authentic specimen.

Anal. Calcd. for $C_{12}H_{14}O_5$: C, 60.50; H, 5.92; OCH_3, 39.08. Found: C, 60.46; H, 5.95; OCH_3, 38.94.

The aqueous layer remaining after the chloroform extractions was placed in the refrigerator and allowed to stand overnight, whereupon reserpic acid hydrochloride crystallized (317 mg.), m.p. 250–253° dec.; methyl ester, m.p. 229–231° dec. The identities of the above compounds were

(10) Since submission of our earlier manuscript on rescinnamine there has appeared a communication by E. Haack, A. Popelak, H. Spingler and F. Kaiser, *Naturwissenschaften*, **41**, 214 (1954), in which the isolation of this alkaloid also is reported.

(11) Georg Cronheim, W. Brown, J. Cawthorne, M. I. Toekes and J. Ungarie, *Proc. Soc. Exp. Biol. and Med.*, **86**, 120 (1954).

(12) Georg Cronheim and M. I. Toekes, *J. Pharmacol. & Exper. Therap.*, in press (1955).

(13) The alkaloids in this extract were present principally as their hydrochlorides.

(13a) Norbert Neuss, Harold E. Bonz and J. W. Forbes, THIS JOURNAL, **76**, 2463 (1954).

(14) Analysis by Elek Microanalytical Laboratories, Los Angeles, Calif.

(15) Potentiometric titration in 75% dimethylformamide–water with 0.01 *N* HCl.

established by mixture melting points, and by comparison of their infrared and ultraviolet spectra with those of authentic samples.

Reconstitution of Rescinnamine.—3,4,5-Trimethoxycinnamic acid[16] (2 g.) was converted to the acid chloride by refluxing for 2.5 hours with thionyl chloride (5 ml.) in benzene (100 ml.). The excess thionyl chloride and benzene were removed *in vacuo* yielding a crystalline residue. Methyl reserpate (1.0 g.) and dry pyridine (50 ml.) was added to the crude acid chloride and the stoppered mixture was agitated on an automatic shaker for 16 hours. At the end of this time, ice (50 g.) was added to decompose the excess acid chloride. The solution was filtered and evaporated to dryness *in vacuo* with the aid of several small additional portions of benzene. The resulting tan colored resin was dissolved in chloroform (100 ml.) and washed successively with equal volumes of dilute hydrochloric acid, dilute aqueous potassium hydroxide and water. The chloroform layer was then taken to dryness and the resulting resinous material was crystallized from benzene (20 ml.) yielding needles (1.10 g.). After several recrystallizations from acetone–water, the sample melted at 237–238° (vac.), $[\alpha]^{24}D$ −95 ± 2 (*c* 1.0 in $CHCl_3$).

(16) K. H. Slotta and H. Heller, *Ber.*, **63**, 3029 (1930).

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LOS ANGELES, CALIFORNIA

[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS, AND THE WELLCOME RESEARCH LABORATORIES]

Pyrimidopteridines by Oxidative Self-condensation of Aminopyrimidines[1,2]

BY E. C. TAYLOR, JR.,[3] HARVEY M. LOUX, ELVIRA A. FALCO AND GEORGE H. HITCHINGS

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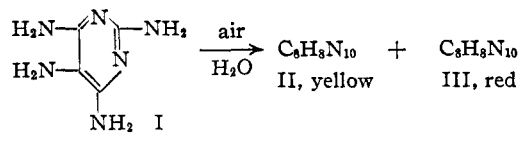
The extremely insoluble, highly fluorescent and deeply colored substances commonly encountered as by-products during the course of reactions involving 4,5-diaminopyrimidines and formerly believed to be amorphous decomposition products of the latter have been found to be pyrimido[5,4-g]- and pyrimido[4,5-g]pteridines formed by oxidative self-condensation of the diaminopyrimidine in the presence of air. A number of examples of the reaction have been given which illustrate its scope and limitations and a mechanism for the conversion has been advanced. The potassium ferricyanide oxidation product of 5-aminouracil (XIII) has been shown to be 2,4,6,8-tetrahydroxypyrimido[4,5-g]pteridine (XII) rather than XIV ("diuracilpyridazine") as previously reported.

4,5-Diaminopyrimidines are commonly used intermediates for the synthesis of purines, pteridines and related condensed pyrimidine systems. The formation of extremely insoluble, highly fluorescent and deeply-colored by-products during these reactions, particularly when carried out in alkaline solution, has been observed frequently, and it has been assumed that these substances were amorphous decomposition products of the diaminopyrimidines. The present paper presents evidence to show that these substances are instead pyrimidopteridines formed by oxidative self-condensation of the diaminopyrimidine in the presence of air.

This investigation was initiated by the observation that a fluorescent, insoluble and deeply-colored substance was formed as a by-product during the course of a synthesis which involved 2,4,5,6-tetraminopyrimidine (I). Trials with various combinations of the components of the original reaction mixture demonstrated that none of the other components was involved and that the product in question must have originated from the tetraminopyrimidine. This conclusion was confirmed by the observation that the same product was formed in 60% yield (based on I) by passing a slow stream of air through a warm aqueous solution of I.

An examination of the ultraviolet absorption spectrum of the new substance showed the presence of intense absorption bands in both the near and far ultraviolet of a character which suggested a relationship to the "bis-alloxazine" of Wieland.[4,5] When the substance was recrystallized from glacial acetic acid, a separation into two isomeric compounds with the empirical formula $C_8H_8N_{10}$ was achieved. Both components were isolated from the recrystallization as their yellow acetates; the major component, which was the more soluble, was obtained from its acetate as a yellow solid (II)



which imparted a strong blue fluorescence to aqueous solutions, while the minor component was obtained from its acetate as a dark red crystalline solid (III) which imparted a greenish-yellow fluorescence to aqueous solutions.

Deamination of the yellow isomer II with sodium nitrite in dilute hydrochloric acid gave a product, $C_8H_4N_6O_4$, which proved to be identical with an authentic sample of "bis-alloxazine," as shown by comparison of both ultraviolet and infrared absorption spectra. "Bis-alloxazine" was first prepared by Wieland in 1940[4] by the condensation of alloxan (V) with 4,5-diaminouracil (VI), and its structure was established as 2,4,5,7-tetrahydroxypyrimido[5,4-g]-pteridine (IV) both by an unequivocal synthesis due to Timmis[6] from barbituric acid (VII) and 6-amino-2,4-dihydroxy-5-nitrosopyrimidine (VIII) and by Taylor, Cain and Loux[5] by

(1) This work was supported in part by a research grant (C-2031-ET) from the National Cancer Institute of the National Institutes of Health, Public Health Service, to the University of Illinois.

(2) Presented before the Division of Organic Chemistry at the 126th Meeting of the American Chemical Society, September 12–17, 1954, New York City.

(3) Frick Chemical Laboratory, Princeton University, Princeton, New Jersey.

(4) H. Wieland, A. Tartter and R. Purrmann, *Ann.*, **545**, 209 (1940).

(5) E. C. Taylor, Jr., C. K. Cain and H. M. Loux, THIS JOURNAL **76**, 1874 (1954).

(6) G. M. Timmis, *Nature*, **164**, 139 (1949).