with diethanolamine and with dichlorodiethylamine to give the corresponding urea derivatives, and also with thiodiglycol to give a product which after treatment with thionyl chloride yields chloroethylthio substituted benzanthranylmethylurethan.

Certain rationalizations are presented with regard to the interest in the β-chloroethyl substituted derivatives as possible agents in a study of chemotherapy of cancer.

CAMBRIDGE 38, MASSACHUSETTS
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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF MERCK & CO., INC.]

Plant Insecticides. I. Ryanodine, A New Alkaloid from Ryania Speciosa Vahl.

BY EDWARD F. ROGERS, FRANK R. KONIUSZY, JOHN SHAVEL, JR., AND KARL FOLKERS

In a survey of plant materials for new insecticides, which has been conducted in coöperation with the Department of Entomology of Rutgers University, it was found that extracts of the stem and root of *Ryania* spp. (family, *Flacourtiaceae*) showed insecticidal activity.[1] Extracts of *Ryania speciosa* Vahl. (*Ryania pyrifera* [L. C. Rich.] Vitt.) were very active and promising.

Entomological tests on stem wood dusts of *Ryania speciosa* Vahl. have been reported by Pepper and Carruth,[2] and other investigators.[3]

Chemical studies of *Ryania* constituents have been reported by Quintero Serra and Gomez,[4] who examined *Ryania speciosa* Vahl. and by Nakarai and Sano,[5] Le Cointe,[6] Bret[7] and Merz,[8] who all worked with root material of *Ryania acuminata* Spruce (*Patrisia acuminata*). These investigators attempted to isolate the principle or principles responsible for the mammalian toxicity of *Ryania* extracts. Various toxic preparations were secured, but these were amorphous and were not analyzed or otherwise characterized. Nakarai and Sano, and Le Cointe and Bret named their toxic fractions "ryanine" and "ryanetine," respectively. It was suggested that these materials were glycosides.

Insecticidal preparations are obtained from the stem and root material of *Ryania speciosa* Vahl. by extraction with water and many organic solvents. Quantitative extraction of the insecticidal principles can be secured by the use of water, methanol or chloroform. Chloroform extraction is much more effective with wet wood than with dry. Thus, in the time required for quantitative extraction of wet wood by chloroform, extraction of dry wood is only about 25% completed. That these methods of extraction are quantitative was proved in two ways. Firstly, bioassay[9] results showed that a constant amount of insecticidal activity was obtained by the three extraction methods; secondly, the marcs obtained were non-insecticidal and subsequent extraction with other solvents yielded non-insecticidal material. The extractives which were secured with methanol, water and chloroform contained considerable amounts of inactive substances. In practice, the chloroform and water extractives are favored for further purification procedures. A concentrated aqueous solution of crude material can be extracted with either ether or amyl acetate to remove the insecticidal principles from the water solution. The distribution coefficients of the insecticidal principles have been determined by bioassay[9] to be approximately 0.6 for ether/water and approximately 7.5 for amyl acetate/water. Alternatively, the active principles in a concentrated chloroform extract can be transferred to water, since the distribution coefficient for water/chloroform is approximately 9.0, and then extracted from the water by ether or amyl acetate. Ether or acetone solutions of the ether and amyl acetate extractives gave crude crystalline material which melted between 140° and 160°. By repeated crystallization of this product from ether, a pure compound was obtained; m. p. 219–220°, $[\alpha]^{25}\text{D} + 26°$ (methanol). This compound has been designated ryanodine. It is highly insecticidal and possesses approximately 700 times the potency of the stem wood of *Ryania speciosa* Vahl.

Ryanodine is neutral to litmus and forms no salts. It does not give precipitates with the common alkaloid reagents, such as the Mayer, Scheibler, Sonnenschein and Wagner reagents. The presence of a pyrrole-like ring system, which may explain the lack of basicity, is suggested by

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(1) Folkers, Rogers and Heal, U. S. Patent 2,400,295, May 14, 1946.

(2) Pepper and Carruth, *J. Econ. Entomol.*, **38**, 59 (1945).

(3) Wheeler and La Plante, *ibid.*, **39**, 211 (1945); Wheeler, *ibid.*, **38**, 281 (1945); Huckett, *ibid.*, **39**, 184 (1946); Bishopp, *ibid.*, **39**, 449 (1946); Dills and Odland, *Vegetable Growers News* (Pennsylvania State College), July, 1946; Dugas and Concienne, *Sugar Cane Investigations in* **1946**, Part I (Louisiana Agricultural Experiment Station); Rainwater and Bondy, *J. Econ. Entomol.*, **40**, 371 (1947); Decker, Apple, Wright and Petty, *ibid.*, **40**, 395 (1947); Bigger, Decker, Wright and Petty, *ibid.*, **40**, 401 (1947); Carruth, *Farm Research* (New York Agricultural Experiment Station), **13**, 11 (1947); Polivka, *Farm and Home Research* (Ohio Agricultural Experiment Station), **33**, 107 (1947); Turner, Connecticut Agricultural Experiment Station Circular 164, April, 1947; Ivy and Ewing, *J. Econ. Entomol.*, **40**, 568 (1947); Ingram, Bynum and Charpentier, *ibid.*, **40**, 779 (1947); Kulash, *ibid.*, **40**, 927 (1947).

(4) Quintero Serra and Gomez, Thesis of R. Quintero Serra, Central University of Venezuela, Caracas, May 15, 1939.

(5) Nakarai and Sano, *J. Pharm. Soc. Japan*, **48**, 157 (1928); *Arch. Pharm.*, **272**, 1 (1934).

(6) Le Cointe, *Bol. Esc. Chim. Ind.* (*Belem*), **1**, 43 (1930).

(7) Bret, *ibid.*, **1**, 48 (1930).

(8) Merz, *Arch. Pharm.*, **268**, 592 (1934).

(9) The bioassay employed measures protection against clothes moth larvae damage. This method was developed by Dr. Ralph E. Heal of these Laboratories.

color tests. The vapors produced by fusion of ryanodine gave a positive pine splinter test. When ryanodine was treated with *p*-dimethylaminobenzaldehyde and sulfuric acid (Van Urk test), a permanent blue color developed. Ryanodine has also been found to give a red color, changing to green-brown with concentrated sulfuric acid and a transient purple-red color with concentrated nitric acid.

Ryanodine is soluble in water, alcohol, acetone, ether and chloroform, with difficultly soluble in benzene, and insoluble in petroleum ether. The distribution coefficient of ryanodine for the system ether/water is 1.3. The ultraviolet absorption spectrum shows a maximum at 2685 Å. ($E_{1}^{1}\%$cm. = 352) in alcohol solution.

A determination of the molecular weight of ryanodine by the Rast method gave a value of 487. The microanalytical data appear to be in agreement with the formulas $C_{25}H_{35}NO_9$ and $C_{26}H_{37}NO_9$, having molecular weights of 493 and 508. Further analytical and degradative evidence is being sought to establish the exact molecular formula of complex alkaloid. The results of functional group analyses indicate that ryanodine contains six or seven active hydrogen atoms, a minimum of three carbon–methyl groups and no acetyl, methoxyl or nitrogen–methyl groups.

Ryanodine appears to be the first characterized alkaloid from a plant of the *Flacourtiaceae* family. Two flacourtiaceous plants, *Gynocardia odorata* and *Pangium edule*, have been reported to contain a cyanogenetic glucoside, gynocardin,[10] $C_{13}H_{19}NO_9$. This report is of interest in view of the observations previously mentioned, that toxic *Ryania* fractions contained glycosides. Ryanodine, however, yielded neither a reducing sugar nor hydrocyanic acid on hydrolysis with dilute acid.

The chloroform extractives, containing the total insecticide activity of *Ryania* wood, has been examined by the Craig distribution technique.[11] A "nine-plate" distribution of the extractives between ether and water was followed by the bioassay procedure. The results, given in Table I, indicate that the insecticidal principles move into the center separatory funnels and that substantial purification can thus be effected. The presence of a principle in *Ryania speciosa* having a very large or very small distribution coefficient for ether–water appears to be unlikely. The distribution of the activity indicates the presence of a principle or principles having a distribution coefficient below unity.

Experimental

Numerous extraction techniques have been applied to plant material of *Ryania speciosa* Vahl. Two typical examples of satisfactory procedure for isolation of ryanodine are as follows:

Water Extraction of Root of *Ryania speciosa* Vahl. (Brooks 15180).[12]—One kilogram of root, ground to approximately 20-mesh, was extracted twice by stirring with 8-l. volumes of boiling water for three-hour periods. The plant material was separated after the extractions by means of a basket centrifuge. The combined extracts were concentrated *in vacuo* to a volume of 500 ml. The concentrate was clarified by centrifuging and then extracted continuously with ether for sixteen hours. The filtered ether extract was concentrated to dryness *in vacuo*, final traces of water being removed by an oil pump. The residue, a light-brown, fluffy solid, weighed 4.1 g. It was redissolved in 250 ml. of ether by refluxing for a short time. The ether solution was concentrated to a 100-volume, then allowed to stand at room temperature until crystallization began (one to two days). The solution was then placed in an icebox for completion of the crystallization. Premature chilling caused precipitation of amorphous material which hindered crystallization. The yield of crude material, which melted at 142–150°, was 1.15 g. By six recrystallizations of the crude material from ether, 180 mg. of pure ryanodine, which melted constantly at 219–220°, was obtained; $[\alpha]^{25}D$ +26° (*c*, 1.02 in methyl alcohol). All samples were dried to constant weight at 100° *in vacuo* before analysis.

Anal. Calcd. for $C_{25}H_{35}NO_9$: C, 60.83; H, 7.15; N, 2.84; mol. wt., 493.5; 7 active H, 1.43; 3(C)-CH_3, 9.14. Calcd. for $C_{26}H_{37}NO_9$: C, 61.52; H, 7.35; N, 2.76; mol. wt., 507.57. Found: C, 61.01, 60.89, 60.78, 61.29, 61.37, 61.22, 61.26 (average 61.14); H, 7.29, 7.41, 7.51, 7.45, 7.26, 7.00, 7.10 (average 7.29); N, 2.74, 2.83, 2.82, 2.79, 2.94 (average 2.82); mol. wt. (Rast), 486.6; active H (hot), 1.21, 1.44, 1.42, 1.18 (average 1.31); (C)-CH_3, 9.10. Methoxyl, N-methyl and acetyl determinations were negative.

Chloroform Extraction of Wet Stem of *Ryania speciosa* Vahl. (Commercial Sample.)—Five hundred grams of stem, ground to approximately 20-mesh, was thoroughly wetted by admixture with 800 ml. of water. The wet wood was placed in a Soxhlet apparatus and extracted with chloroform for twenty-four hours. The chloroform extract was concentrated to a volume of 750 ml., then extracted with four 300-ml. volumes of water. The combined aqueous extracts were extracted with four 200-ml. volumes of amyl acetate. The amyl acetate extract was concentrated to dryness *in vacuo*, giving 1.925 g. of a light-brown powder. Crystallization of this material from concentrated acetone solution yielded 893 mg. of crude alkaloid melting at 155–160°, which gave 143 mg. of pure ryanodine after five recrystallizations from ether.

Distribution Study of a Chloroform Extractive.—The chloroform extractive from 100 g. of stem material, weighing 1.321 g., was placed in a separatory funnel and shaken with 50 ml. of wet ether and 50 ml. of ether-saturated water until entirely dissolved. The aqueous layer was then transferred to a second separatory funnel and shaken with 50 ml. of wet ether, and so on, until a "nine-plate" distribution[11] was completed. The contents of the nine separatory funnels were evaporated to dryness *in vacuo* and weighed. The residues secured were assayed. The weights and percentages of total insecticidal principles of the contents of the nine funnels are given in Table I.

TABLE I

DISTRIBUTION OF CHLOROFORM EXTRACTIVE

Funnel no.	Wt., mg.	Total potency, %
1	278	1.3
2	37	1.8
3	58	4.9
4	97	17.6
5	157	22.0
6	162	27.0
7	140	17.1
8	122	6.9
9	207	1.8

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[10] Power and Lees, *J. Chem. Soc.*, **87**, 349 (1905).

[11] Craig, *J. Biol. Chem.*, **150**, 33 (1943); Craig, Golumbic, Mighton and Titus, *ibid.*, **161**, 321 (1945).

[12] The botanical specimens corresponding to this plant sample are deposited in the New York Botanical Gardens.

Acknowledgment.—We are indebted to Mr. B. A. Krukoff for the task of obtaining the plant materials, for the determinations of plants and for his suggestions and advice on botanical matters. We wish to express our appreciation to Dr. Ralph E. Heal for the bioassays. The coöperation of Messrs. Douglass Hayman, R. N. Boos and associates on microanalyses was very helpful.

Summary

An insecticidal alkaloid, ryanodine, tentatively formulated as $C_{25}H_{35}NO_9$ or $C_{26}H_{31}NO_9$, has been isolated from root and stem material of *Ryania speciosa* Vahl.

RAHWAY, N. J. RECEIVED APRIL 13, 1948

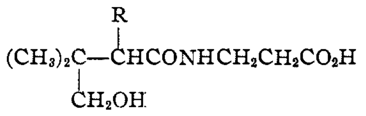
[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF MERCK & CO., INC.]

An Amino Analog of Pantothenic Acid

BY FREDERICK W. HOLLY, RODERICK A. BARNES,[1a] FRANK R. KONIUSZY AND KARL FOLKERS

An amino analog of pantothenic acid, as an ethyl ester, has been synthesized. This analog, ethyl DL-β'-(α-amino-β,β-dimethyl-γ-hydroxybutyrylamino)-propionate, showed no vitamin activity when assayed for pantothenic acid activity for *Lactobacillus arabinosus* or when tested in pantothenic acid deficient rats. In the presence of pantothenic acid, however, the analog had vitamin activity for *Lactobacillus arabinosus.*

Of the many analogs of pantothenic acid (I) which have been prepared,[1] the amino analog, β'-(α-amino-β,β-dimethyl-γ-hydroxybutyrylamino)-propionic acid (II), has not been included.



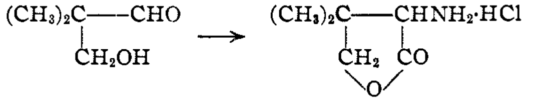
$$\begin{array}{l} \qquad\quad R \\ (CH_3)_2C—CHCONHCH_2CH_2CO_2H \\ \qquad CH_2OH \end{array}$$

I. R = OH

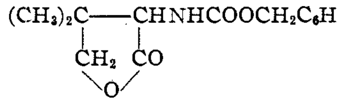
II. R = NH_2

It was of interest to synthesize and test biologically this amino analog, because the compound possesses structural relationship to the naturally occurring α-amino acids.

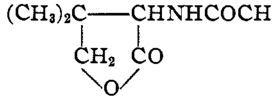
α,α-Dimethyl-β-hydroxypropionaldehyde[2] (III) was converted into DL-α-amino-β,β-dimethyl-γ-butyrolactone hydrochloride[2a] (IV) by a modified Strecker reaction.[3]



III IV



V



VI

The N-carbobenzoxy derivative of the amino lactone (V) was prepared by reaction of the amino lactone hydrochloride with carbobenzoxy chloride in diethylaniline–benzene solution. The N-acetyl derivative of the amino lactone (VI) was obtained by reaction of the amino lactone hydrochloride with acetic anhydride in pyridine solution, acetyl chloride in benzene solution, or with acetyl chloride in benzene–dioxane in the presence of barium carbonate.

Condensation of α-carbobenzoxyamino-β,β-dimethyl-γ-butyrolactone with β-alanine ethyl ester yielded ethyl DL-β'-(α-carbobenzoxyamino-β,β-dimethyl-γ-hydroxybutyrylamino)-propionate (VII), which was obtained as an oil. The carbobenzoxy group of the β-alanine derivative (VII) was readily removed by hydrogenolysis of the compound over a palladium–charcoal catalyst[4] in ethanol–hydrochloric acid solution. This reaction yielded the hydrochloride of the amino analog of pantothenic acid as an ethyl ester (VIII). Since this ethyl ester (VIII) was an oil, it was characterized further by reaction with acetic anhydride in pyridine to give a crystalline diacetyl derivative (IX). This diacetyl derivative (IX) had the same melting point and infrared absorption spectrum as another sample of the diacetyl derivative which had been synthesized by alternative reactions.

The alternative synthesis of the diacetyl derivative (IX) started with DL-α-acetylamino-β,β-dimethyl-γ-butyrolactone (VI). Reaction of this acetylamino lactone with β-alanine ethyl ester gave ethyl DL-β'-(α-acetylamino-β,β-dimethyl-γ-hydroxybutyrylamino)-propionate (X) and then acetylation of this compound gave ethyl DL-β'-(α-acetylamino-β,β-dimethyl-γ-acetoxybutyrylamino)-propionate (IX).

Dr. H. B. Woodruff of the Microbiological Department has kindly assayed the amino analog

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[1] Many references to the literature in this field have been cited by Cheldelin and Schink, THIS JOURNAL, **69**, 2625 (1947). See also McIlwain and Hughes, *Biochem. J.*, **39**, 133 (1945).

[2] Stiller, Harris, Finkelstein, Keresztesy and Folkers, THIS JOURNAL, **62**, 1785 (1940).

[2a] The acid corresponding to this lactone has now been reported by Ackermann and Kirby (*J. Biol. Chem.*, **175**, 483 (1948)) as possibly being a naturally occurring amino acid (note added in proof).

[3] Clarke and Bean, "Organic Syntheses," Coll. Vol. II, John Wiley & Sons, Inc., New York, N. Y., 1943, p. 29.

[4] Mozingo, "Organic Syntheses," Vol. XXVI, John Wiley & Sons, Inc., New York, N. Y., 1946, p. 78, procedure B.