obtained. The data did, however, indicate that the titrating material was located primarily in fraction 1. The absence of free nucleosides was confirmed both by paper chromatography[10] and by paper ionophoresis. The absence of nucleoside-5'-phosphates was demonstrated using the paper ionophoretic technique previously described. From this one must conclude that there are compounds present in ribonucleic acid which are as yet uncharacterized.

Studies on Fraction 2.—The chromatographic results suggest that this fraction is composed of substances of greater complexity than mononucleotides. It gives a negative test for desoxyribose[15] and is therefore apparently derived from ribonucleic acid. When resubmitted to chromatography using solvent 1, this fraction separates into five major spots and one minor spot with reference to relative concentration (Fig. 2B). The six spots were eluted with water and the ultraviolet absorption curves run for each, using corresponding paper blanks (Fig. 3).

Samples of each of the six spots were hydrolyzed, chromatographed and analyzed according to the method of Smith and Markham.[4d] The molar ratios of the six spots are given in Table II. The curves calculated from these data when compared with the experimental curves were in close agreement.

TABLE II

NUCLEOTIDE COMPOSITION OF THE COMPOUNDS IN FRACTION 2

	Molar ratios Spot					
Nucleotide acid	1	2	3	4	5	6
Guanylic	3	7	10	1	..	..
Adenylic	..	..	2	4	19	1
Cytidylic	..	1	1	3	1	..
Uridylic	1	2	1	..	..	..

The unknown spot appearing between adenylic acid and guanylic acid on a formate (pH 3.3) ionophoretic separation of total alkaline hydrolysates, when eluted and resubmitted to chromatography in solvent 1 moved with the same R_f as Spot 6 of fraction 2.

This small amount of fraction 2 present in the total alkaline hydrolysates of ribonucleic acid should not detract from its significance and importance in the theory and scope of nucleic acid chemistry. The results presented here not only cast doubt upon the quantitativeness of alkaline hydrolysis to form mononucleotides, but also indicate the future trend in the investigation of the chemical hydrolysis products of ribonucleic acids, that of studying the non-mononucleotide fraction of the hydrolysate which contains linkages stable to alkali at room temperatures.

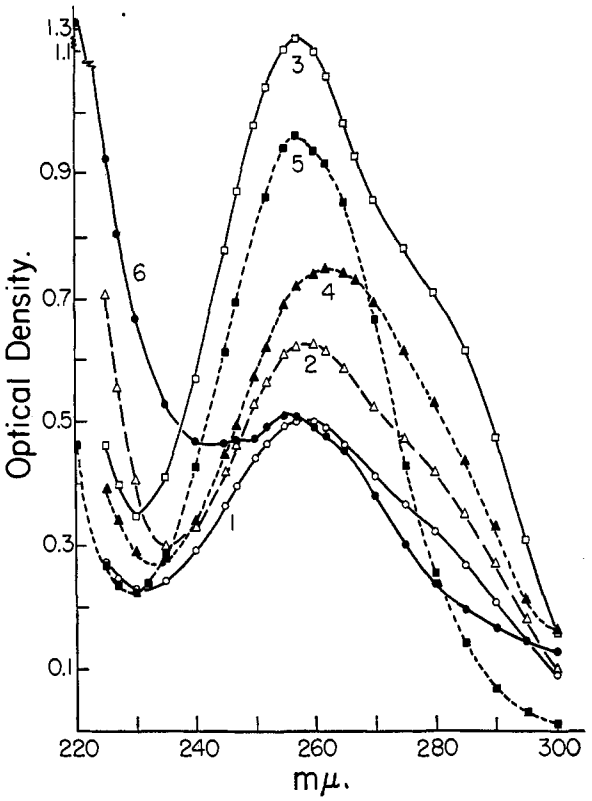


Fig. 3.—Ultraviolet absorption curves of the polynucleotides. Numbers refer to the various substances in fraction 2.

BERKELEY, CALIFORNIA

[CONTRIBUTION FROM RIKER LABORATORIES, INC.]

Alkaloids of *Veratrum eschscholtzii* Gray. I. The Glycosides*

BY M. W. KLOHS, M. D. DRAPER, F. KELLER, W. MALESH AND F. J. PETRACEK

RECEIVED DECEMBER 8, 1952

Isorubijervosine ($C_{33}H_{53}O_7N$), a new glucosidic alkaloid, as well as the known glucosides, pseudojervine and veratrosine, has been isolated from *Veratrum eschscholtzii* Gray. On acid hydrolysis, isorubijervosine yielded the aglycone isorubijervine, and D-glucose. Isorubijervosine was shown to be 3(β]-D-glucosyl-Δ^5-solanidene-18-ol.

Isorubijervosine, a new glucosidic alkaloid, has been isolated from the hitherto uninvestigated species, *Veratrum eschscholtzii* Gray, as well as the known glucosides, pseudojervine and veratrosine.

Pseudojervine was originally isolated by Wright and Luff,[1] from *Veratrum viride* Ait. and *Veratrum album* L. The empirical formula ($C_{29}H_{43}O_7N$) advanced by these workers was later revised to $C_{33}H_{49}O_8N$ by Poethke[2]; however, the glycosidic character of this alkaloid was not revealed until the work of Jacobs and Craig[3] showed it to be the glucoside of the alkamine jervine. The latter investigators also succeeded in isolating veratrosine[3] ($C_{33}H_{49}O_7N$), and showed it to be the glucoside of the alkamine, veratramine.

After investigating the hypotensively active amorphous bases[4] of *Veratrum eschscholtzii* Gray, our attention was directed to the more hydrophilic fractions herein designated as II and IV.[5] On dissolving fraction I in boiling ethanol, pseudojervine rapidly separated as an insoluble white powder. The mother liquors were evaporated to dryness and

* Presented before the division of Medicinal Chemistry of the American Chemical Society, Los Angeles, Calif., March 15–19, 1953.

(1) C. R. A. Wright and A. P. Luff, *J. Chem. Soc.*, **35**, 405 (1879).

(2) W. Poethke, *Arch. Pharm.*, **276**, 170 (1938).

(3) W. A. Jacobs and L. C. Craig, *J. Biol. Chem.*, **155**, 565 (1944).

(4) The hypotensively active alkaloids have been described in a preliminary communication; M. W. Klohs, F. Keller, S. Koster and W. Malesh, THIS JOURNAL, **74**, 1871 (1952).

(5) Fraction IV when treated in the same manner as Fraction II yielded additional quantities of isorubijervosine and pseudojervine.

chromatographed on an alumina column. The more polar solvents eluted a mixture which on fractional crystallization from methanol, yielded veratrosine and a new alkaloid, isorubijervosine, m.p. 279–280°, $[\alpha]^{24}D$ −20 ± 2° (*c* 1.45 in py.). The formulation $C_{33}H_{53}O_7N$ was derived by analysis of the base and its pentaacetyl derivative. The empirical formula, infrared spectrum (Fig. 1) and sparing solubility of the alkaloid strongly suggested its glycosidic character, which was further supported by a positive Fehling test after acid hydrolysis. The products of hydrolysis were shown to be isorubijervine[6] and D-glucose. Isorubijervine was identified by its physical constants, analysis, infrared spectrum (Fig. 1) and by conversion to diacetylisorubijervine. D-Glucose was identified by its phenylosazone and 2,4-dinitrophenylhydrazone derivatives. The composite formula is thus in agreement with that formulated on the basis of analytical data.

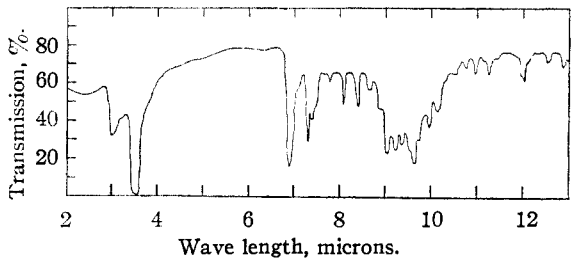
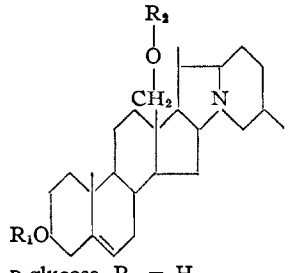


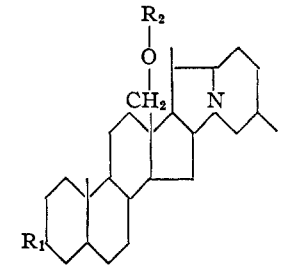
Fig. 1.—Infrared spectrum: isorubijervosine in Nujol mull.

The 3(β)-hydroxyl was thought to be the site of attachment of D-glucose on isorubijervine, since isorubijervosine, in contrast to the aglycone, failed to form an insoluble digitonide. The recent structural elucidation of isorubijervine as Δ^5-solanidene-3(β),18-diol[7–10] suggested the conversion of isorubijervosine (I) to solanidane-3-one-18-acetate (V) as a means of confirming the site of conjugation.

This was readily accomplished by first protecting the free hydroxyl on the steroid nucleus of isorubijervosine (I) by reaction with an excess of acetic anhydride, yielding pentaacetylisorubijervosine (II); the latter compound was then refluxed with 2% hydrochloric acid in absolute ethanol, selectively cleaving the glucosidic linkage. The mono-



I, R_1 = D-glucose, R_2 = H
II, R_1 = Tetraacetyl-D-glucose, R_2 = CH_3CO–
III, R_1 = H, R_2 = CH_3CO–



IV, R_1 = OH, R_2 = CH_3CO–
V, R_1 = O, R_2 = CH_3CO–

acetylisorubijervine (III) thus obtained was hydrogenated over PtO_2 and the resulting dihydro compound (IV) was oxidized with chromic acid to yield solanidane-3-one-18-acetate. The physical constants, analysis and infrared spectrum of the latter were in agreement with an authentic sample. The structure of isorubijervosine has thus been established as 3-(β)-D-glucosyl-Δ^5-solanidene-18-ol.

Experimental[11]

Extraction of Roots and Rhizomes of Veratrum Eschscholtzii.—Ground dried roots and rhizomes of *Veratrum eschscholtzii*[12] (45 kg.) were extracted with four 200-l. portions of chloroform–ammonium hydroxide (1 l. of ammonium hydroxide per 50 kg. of chloroform) over a period of 36 hours. The extracts were drained through filter pads into a stainless steel vacuum still and concentrated to a combined volume of 80 l. at 21–23 inches gage pressure and then further concentrated in glass stills at 100–120 mm. pressure to 5.5 kg. of a brown, viscous liquid. The concentrate was poured with stirring into aqueous 5% acetic acid (27 l.) and the resulting tarry materials were removed by filtration. The filtrate was cooled to 6° in a jacketed tank, and 6 *N* sodium hydroxide (4 l.) added to *p*H 7.2 (maximum temperature during this step was 14°). The free bases were extracted immediately with two 10-l. and two 5-l. portions of chloroform. The combined extracts were dried over anhydrous sodium sulfate and concentrated to dryness at 100–120 mm. pressure, yielding a tan resin (1530 g.) designated fraction I. The aqueous residue after chloroform extraction at *p*H 7.2 was adjusted to *p*H 10.9 with 6 *N* sodium hydroxide and extracted immediately with two 10-l. and two 5-l. portions of chloroform. The combined extracts were dried over anhydrous sodium sulfate and concentrated to dryness at 100–120 mm. pressure yielding a tan resin (90 g.) designated as fraction II.

A 500-g. portion of fraction I was dissolved in 5% aqueous acetic acid (15 l.) and a solution of ammonium sulfate (750 g. in 1.1 l. water) was added slowly and then stirred for 1 hour. The precipitated fraction (170 g.) thus obtained on filtration consisted mainly of secondary alkaloids and was designated fraction III. The filtrate was cooled and 6 *N* sodium hydroxide added to *p*H 7.3 (maximum temperature during this step was 11°). The free bases were immediately extracted with thirteen 8-l. portions of chloroform. The combined dried chloroform extracts were concentrated to 5 l. in a stainless steel still at 21–23 inches gage pressure and then concentrated to dryness in glass stills at 100–120 mm. pressure, yielding 327 g. of free bases. The free bases (300 g.) were dissolved as much as possible in benzene (2 l.) by stirring for 1 hour at room temperature and the insoluble portion was removed by filtration. The solids (fraction IV) were washed with benzene (1 l.), and worked up for pseudojervine and isorubijervosine as described below for fraction II. The filtrate and wash were combined and concentrated to dryness at 100–120 mm. pressure yielding the amorphous bases (235 g.) designated fraction V.

The Isolation of Isorubijervosine, Pseudojervine and Veratrosine from Fraction II.—The resinous material representing fraction II (40 g.) was dissolved in ethanol (200 ml.)

(6) W. A. Jacobs and L. C. Craig, *J. Biol. Chem.*, **148**, 41 (1943).
(7) Y. Sato and W. A. Jacobs, *ibid.*, **191**, 63 (1951).
(8) W. Rigby and D. Burn, *Chemistry and Industry*, **27**, 668 (1952).
(9) Frank L. Weisenborn and Diana Burn, Abst. 122nd Meeting A. C. S., Atlantic City, N. J., September, 1952, 23L.
(10) S. W. Pelletier and Walter A. Jacobs, THIS JOURNAL, **74**, 4218 (1952).

(11) All melting points are corrected. They were taken in evacuated capillaries using a Hershberg melting point apparatus with total immersion N. B. S. calibrated thermometers.
(12) The roots and rhizomes of *Veratrum eschscholtzii* were gathered during the summer of 1950 in Alaska.

and heated to boiling on the steam-bath. After several minutes, a white powder began to separate; the solution was heated for another 15 minutes and then filtered while hot. The white powder thus obtained (5.0 g.) was shown to be pseudojervine by comparison with an authentic sample. The filtrate was evaporated to dryness under vacuum and dissolved in benzene–chloroform 1:1 (200 ml.). The solution was then chromatographed on an alumina column (800 g.) using the series benzene–chloroform, chloroform–methanol and methanol–5% aqueous acetic acid for elution. From the fractions eluted with chloroform–5–10% methanol, a small amount of an unidentified alkamine was obtained which on recrystallizing from chloroform–methanol yielded needles, m.p. 243°, $[\alpha]^{24}D$ −109 ± 2° (*c* 0.54 in 95% ethanol). The fractions eluted with MeOH–5–15% of 5% aqueous acetic acid were concentrated under vacuum to remove the methanol, made basic with sodium hydroxide and extracted with chloroform until the mother liquors gave a negative test with Wagner reagent. The chloroform extracts were dried over anhydrous sodium sulfate and then evaporated to dryness under vacuum. The residue (5.0 g.) was dissolved in methanol and concentrated on the steam-bath to effect crystallization. Crude isorubijervosine crystallized as fine needles (total yield 2.0 g.). On diluting the mother liquor with water to faint turbidity and allowing the solution to stand, a crop of fine, woolly needles (100 mg.) was obtained, which was identified as veratrosine, m.p. 236–239° (open tube), 276–278° (evac. tube), $[\alpha]^{24}D$ −55 ± 2° (*c* 0.94 in ethanol–chloroform 1:1). Jacobs and Craig[3] report a melting point of 242–243° (open tube), $[\alpha]^{25}D$ −53° (*c* 0.255 in ethanol–chloroform 1:1). The infrared and ultraviolet spectra were identical with those of an authentic sample of veratrosine from *Veratrum viride* Ait.

Isorubijervosine (I).—The crude isorubijervosine (2.0 g.) was recrystallized several times by dissolving with refluxing in methanol (120 ml.) and concentrating on the steam-bath until crystallization commenced. Isorubijervosine was recovered as fine needles, m.p. 279–280°; $[\alpha]^{24}D$ −20.2 ± 2 (*c* 1.45 in py.), −20 ± 2° (*c* 0.43 in EtOH). For analysis the sample was dried at 150° (2 mm.) to constant weight.

Anal. Calcd. for $C_{33}H_{53}O_7N$: C, 68.83; H, 9.28; equiv. wt., 575.76; N, 2.43. Found: C, 68.72; H, 9.38; equiv. wt., 588; N, 2.73.

Isorubijervosine Pentaacetate (II).—Isorubijervosine (100 mg.) was refluxed for 2 hours with acetic anhydride (5 ml.), and then evaporated to dryness under vacuum. The residue was dissolved in water (5 ml.), made basic to *p*H 7.3 with a saturated sodium carbonate solution and extracted 3 times with chloroform (10-ml. portions). The chloroform extracts were dried over anhydrous sodium sulfate and concentrated to dryness under vacuum. The residue was dissolved in acetone (5 ml.) and diluted to faint turbidity with water. Rapid crystallization yielded fine needles (80 mg.), m.p. 202–203°, $[\alpha]^{24}D$ −5.9 ± 2 (*c* 1.0 in $CHCl_3$). For analysis the sample was dried at 120° (2 mm.) to constant weight.

Anal. Calcd. for $C_{43}H_{63}O_{12}N$: C, 65.71; H, 8.27; acetyl, 27.4. Found: C, 65.91; H, 8.23; acetyl, 27.4.

Hydrolytic Cleavage of Isorubijervosine to Isorubijervine and D-Glucose.—Isorubijervosine (1.0 g.) was refluxed in 2% aqueous hydrochloric acid (70 ml.) for 2.5 hours. The solution was made basic to *p*H 7.2 with 10% aqueous sodium hydroxide and extracted with four 20-ml. portions of chloroform. The chloroform extracts were dried over anhydrous sodium sulfate and evaporated to dryness under vacuum. The residue was crystallized from acetone yielding prisms (0.52 g.), m.p. 240–242°, $[\alpha]^{24}D$ +7.7 ± 2° (*c* 0.92 in abs. EtOH). The melting point and optical rotation of isorubijervine[6] has been reported as 235–237° (uncor.), $[\alpha]^{25}D$ +6.5° in EtOH. The infrared spectrum (Fig. 1) was identical with that of an authentic sample of isorubijervine. For analysis, the sample was dried at 140° (2 mm.) to constant weight.

Anal. Calcd. for $C_{27}H_{43}O_2N$: C, 78.48; H, 10.49. Found: C, 78.77; H, 10.38.

The diacetate of the isorubijervine obtained from the hydrolysis was prepared in the same manner as previously described for the pentaacetate of isorubijervosine, yielding needles, m.p. 202°; for analysis, the sample was dried at 110° (2 mm.) to constant weight.

Anal. Calcd. for $C_{31}H_{47}O_4N$: C, 74.81; H, 9.52. Found: C, 75.17; H, 9.57.

Isorubijervine diacetate prepared from an authentic sample of isorubijervine melted at 202° and a mixed melting point with the above sample gave no depression.

The aqueous solution remaining after removal of isorubijervine from the hydrolysis mixture was carefully neutralized with dilute hydrochloric acid and concentrated to dryness under vacuum yielding a white residue. The sugar was separated from the sodium chloride by extracting the residue with four 20-ml. portions of hot ethanol. The combined ethanol extracts were evaporated to dryness.

One half of the sugar residue thus obtained was used to prepare the phenylosazone, yielding bright yellow needles, m.p. 205–206°. This is in agreement with that reported for the osazones of the three isomeric hexoses, D-glucose, D-mannose and D-fructose.

The second half of the residue was treated overnight with 2,4-dinitrophenylhydrazine[13-14] yielding needles, m.p. 124–126° with preliminary softening. The melting point of the 2,4-dinitrophenylhydrazone of D-glucose has been reported as 122–124°. A mixed melting point with an authentic sample gave no depression.

Isorubijervine-18-acetate (III).—Isorubijervosine pentaacetate (2 g.) was refluxed with a 2% solution of hydrochloric acid in absolute ethanol (200 ml.) for 1 hour and 15 minutes. The solution was cooled, adjusted to a *p*H of 6.5 with 6 *N* sodium hydroxide and concentrated to approximately 50 ml. under vacuum. Water (100 ml.) was added and the *p*H adjusted to 7.3 with 6 *N* sodium hydroxide and extracted with four 30-ml. portions of chloroform. The chloroform extracts were dried over anhydrous sodium sulfate and concentrated under vacuum to 30 ml. The concentrated extract was then chromatographed on acid-washed alumina using chloroform as the eluting solvent. The first 400 ml. of eluant yielded the monoacetate (0.6 g.) followed by isorubijervine in the next 500 ml. The monoacetate crystallized from acetone; m.p. 219°, $[\alpha]^{24}D$ −0.46 ± 2° (*c* 1 in $CHCl_3$), −2.3 ± 2° (*c* 1.0 in py.).

Dihydroisorubijervine-18-acetate (IV).—Isorubijervine-18-acetate (0.5 g.) was hydrogenated in a solution of 1.5% acetic acid in ethanol (15 ml.) with platinum oxide catalyst (0.2 g.). The hydrogenation was complete after two hours and the catalyst removed by filtration. The filtrate was diluted with water (100 ml.), made basic with 6 *N* sodium hydroxide to *p*H 7.3, and extracted with chloroform. The chloroform extracts were dried over anhydrous sodium sulfate and evaporated to dryness. The residue crystallized from acetone yielding heavy needles (0.4 g.); m.p. 190° with preliminary sintering, $[\alpha]^{24}D$ −22 ± 2° (*c* 1.0 in py.). Sato and Jacobs[7] reported a melting point of 179–182°.

Solanidane-3-one-18-acetate (V).—Dihydroisorubijervine-18-acetate (200 mg.) was oxidized according to the method of Sato and Jacobs.[7] The oxidation product when crystallized from acetone yielded needles (100 mg.), m.p. 179°, −56 ± 2° (*c* 1.0 in py.). Sato and Jacobs reported a melting point of 174–177°. The sample gave no depression when admixed with the acetate derivative prepared from an authentic sample of solanidane-3-one-18-ol kindly supplied by Dr. Frank L. Weisenborn and Diana Burn. The infrared spectra and optical rotation in pyridine were identical. For analysis, a sample was dried at 110° (2 mm.) to constant weight.

Anal. Calcd. for $C_{29}H_{45}O_3N$: C, 76.44; H, 9.96. Found: C, 76.38; H, 9.97.

Acknowledgments.—The authors are indebted to Dr. Adalbert Elek for the micro analyses, to C. H. Stimmel and E. Honey for the optical rotations, infrared spectra and equivalent weight determinations, and to H. DeVorkin and P. Goble for processing additional quantities of isorubijervosine.

LOS ANGELES, CALIF.

(13) E. Alon Lloyd and David G. Doherty, THIS JOURNAL, **74**, 4214 (1952).

(14) E. Glaser and N. Zuckerman, *Z. physiol. Chem.*, **167**, 37 (1927).