When recrystallized to constant melting point from absolute methanol, the compound sintered at 171° and melted at 172-173.5°.

Anal. Calcd. for $C_{13}H_{17}ON_2ClS$: N, 9.83; S, 11.22. Found: N, 9.83; 9.82; S, 11.25; 11.20; 11.10

Summary

6-Quinolyl *p*-nitrophenyl sulfide and sulfone have been prepared by application of the Skraup synthesis.

By the interaction of the sodium salts of appropriate substituted thiophenols and *p*-acetamidobenzenesulfinic acid with the labile chlorine atom of 5-nitro-6-chloroquinoline or 5-nitro-6-chloro-8-amino-(or acetamido-)-quinoline, the synthesis of a series of substituted 6-quinolyl sulfides and sulfones has been effected.

Some *p*-aminophenyl derivatives containing the sulfone group in an alkyl "side-chain" have been prepared.

In connection with studies on experimental avian malaria, γ-(6-methoxy-8-quinolylamino)-propyl mercaptan hydrochloride has been synthesized.

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN COMPANY]

The Formula of Zygadenine

BY F. W. HEYL AND M. E. HERR

A number of years ago one of us isolated from Death Camas leaves a crystalline alkaloid[1] which belongs in the veratrine group.[2] A year or more later a further quantity of this alkaloid, which we called zygadenine, was prepared in this Laboratory. It was subjected to both acid and alkaline hydrolysis but neither carbohydrate nor angelic acid could be detected and the basic fraction failed to crystallize.

More recently the work of Jacobs and others has brought an orderly system to the consideration of this class of alkaloids.[3]

With this highly detailed development in the chemistry of the veratrine alkaloids it did not appear difficult to restudy zygadenine and more clearly fit it into the systematized knowledge in this field. The data in the experimental part indicate that zygadenine has the formula $C_{27}H_{43}O_7N$. It belongs in the series of alkamines which Jacobs and Craig have tabulated as follows: veratramine, $C_{27}H_{39}O_2N$; rubijervine and isorubijervine, $C_{27}H_{43}O_8N$; jervine, $C_{27}H_{39}O_3N$; an unnamed alkaloid, $C_{27}H_{41}O_4N$, from veratrum viride[4]; cevine and isogermine, $C_{27}H_{43}O_8N$; and protoverine, $C_{27}H_{43}O_9N$.

Zygadenine does not form a nitroso compound and is a tertiary base. It appears to be a new alkamine closely related to cevine and germine but containing one less oxygen. The specific rotation of zygadenine differs from those of cevine (−17.52°) and of germine (+5.0°) but it closely approaches that of isogermine (−46.5°).[5] The latter melts above 245° while zygadenine melts at 201–204°.

Experimental[6]

Preparation of Crude Alkaloidal Fractions.—Thirteen and six-tenths kilograms of the dried Death Camas leaves were exhausted with 95% alcohol and the percolate was concentrated to about 6 l. The concentrate was diluted with about 12 l. of water in which 60 g. of tartaric acid had been dissolved. The precipitated resin was removed, the clear solution rendered alkaline with ammonia and the crude alkaloids extracted with chloroform.

The alkaloids were re-extracted with 5% tartaric acid solution and reprecipitated with ammonia. The precipitate "A" (43.6 g.) was filtered, washed with water and dried *in vacuo*. An additional 22.9 g. was extracted from the ammoniacal filtrate with chloroform (fraction "B") so that the total yield of crude alkaloid was equivalent to 0.49% of the dried leaves.

Properties of Fraction "A" above.—When 1.0 g. was saponified with sodium hydroxide in methanol by the method described below, there resulted upon steam distillation of the "volatile acids" 3 thirty-minute distillates which required, respectively, 2.94, 2.4 and 1.9 ml. of 0.05 *N* alkali, from which it may be concluded that fraction "A" contained not more than 20% of esterified alkaloid. In fact this fraction was largely zygadenine and was the source of its preparation in the previous publication.[1]

Zygadenine.—The crude alkaloid "B" served to prepare zygadenine. It was dissolved in boiling alcohol and 17.5 g. of the alcohol addition product crystallized. Fractional crystallization from alcohol yielded four top fractions weighing, respectively, 7.0 g., 6.7 g., 2.1 g., and 0.6 g. The first fraction upon analysis showed the presence of 11.4% alcohol of crystallization. It was dried at 100° *in vacuo* and crystallized from benzene when $[\alpha]^{24}_{D}$ −45° in chloroform. It melted at 201–204°.

Anal. Calcd. for $C_{27}H_{43}O_7N$: C, 65.7; H, 8.8; N, 2.84. Found: C, 65.9, 66.1, 66.1; H, 8.50, 8.75, 8.76; N, 3.17, 2.97, 2.92.

The first three fractions were combined, freed from alcohol and systematically fractionated from benzene. A top fraction weighing 9.88 g. was again crystallized from benzene. It weighed 4.23 g. and upon analysis showed no differences from those reported above. Despite repeated crystallization the carbon content remained on the high side of that required by the steroid formula.

The alkaloid contained neither methoxyl nor N-CH_3 groups. It did not precipitate with digitonin and failed to give any coloration with a 90% solution of trichloroacetic acid.

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(1) F. W. Heyl, F. E. Hepner and S. K. Loy, THIS JOURNAL, **35**, 258 (1913).

(2) P. H. Mitchell and G. Smith, *Am. J. Physiol.*, **28**, 318 (1911).

(3) F. C. Uhle and W. A. Jacobs, *J. Biol. Chem.*, **160**, 243 (1945); W. A. Jacobs and L. C. Craig, *ibid.*, **155**, 565 (1944); **160**, 564 (1945); **170**, 635 (1947); W. Poethke, *Arch. Pharm.*, **275**, 571 (1937); **276**, 179 (1938).

(4) W. A. Jacobs and L. C. Craig, *J. Biol. Chem.*, **160**, 555 (1945); **149**, 451 (1943).

(5) L. C. Craig and W. A. Jacobs, *ibid.*, **148**, 60 (1943).

(6) We wish to acknowledge with thanks the coöperation of the Upjohn microanalytical group.

The alkaloid separated from methanol with 10.24% alcohol, the calculated value for $C_{27}H_{43}O_7N \cdot 2CH_3OH$ requiring 11.50%. As stated above, the crystals which separated from ethanol contained 11.40% alcohol whereas the calculated value for $C_{27}H_{43}O_7N \cdot 1.5C_2H_5OH$ requires 12.18%. The solubility of the pure alkaloid in water at 4° amounted to 0.84 g. per 100 ml. Boiling acetone dissolved approximately 3.63 g. per 100 ml. of solvent from which it crystallized in transparent stout needles.

Molecular Weight by Titration.—This value, determined by ebullioscopic methods, was previously found to vary between 661 and 725.[1] We now consistently find that the molecular weight found by titration is 510 and approximates that required by a steroid alkaloid.

Anal. 0.5372 g. alkaloid in alcohol required 21.25 ml. of 0.05 *N* sulfuric acid, using methyl red. Found: mol. wt., 510.4.

Zygadenine Sulfate.—The titration solution was evaporated to a small volume at 35° and the residue dissolved in boiling methanol. The sulfate crystallized in prisms which decomposed at 237–242°.

Anal. Calcd. for $(C_{27}H_{43}O_7N)_2 \cdot H_2SO_4$: S, 2.95. Found: S, 2.6.

Zygadenine Hydrochloride.—When 0.27 g. was dissolved in a small excess of 0.1 *N* hydrochloric acid (7 cc.) and allowed to evaporate to dryness *in vacuo* at room temperature the resulting hydrochloride became yellow and then tan colored and melted unsatisfactorily at 227–230° with signs as low as 220°. This salt was prepared in pure form by exactly neutralizing the alkaloid in methyl alcohol, concentrating *in vacuo* to a small volume and precipitating the product with ether in three fractions. The top fraction was washed with alcohol–ether (20:80) and ether. The decomposition point was 231–234°. It lost 4.7% when dried *in vacuo* at 100° (calcd. for $C_{27}H_{43}O_7N \cdot HCl \cdot 1.5H_2O$: H_2O, 4.7).

Anal. Calcd. for $C_{27}H_{43}O_7N \cdot HCl$: Cl, 6.65. Found: Cl, 6.46, 6.64, 6.87.

Zygadenine Gold Chloride.—Zygadenine (0.23 g.) was dissolved in a slight excess of 0.1 *N* hydrochloric acid and a slight excess of gold chloride solution was added. There was an immediate crystalline precipitate. After standing the needles were filtered and weighed 0.36 g.; theory requires 0.39 g. The gold salt decomposes chiefly at 140–165° but cannot be said to have any definite decomposition point. These crystals lost 3.48% in weight when dried at 80°. (Calcd. for $C_{27}H_{43}O_7N \cdot HCl \cdot AuCl_3 \cdot H_2O$: H_2O, 3.1.)

Anal. Calcd. for $C_{27}H_{43}O_7N \cdot HCl \cdot AuCl_3$: C, 39.0; H, 5.3; Au, 23.65. Found: C, 39.3; H, 5.5; Au, 23.50, 23.42.

Acetylzygadenine.—The alkaloid was acetylated by the method described by Poethke for germine.[3] The acetate was highly crystalline and melted at 265–270° (dec.). When the acetyl value was determined by the method of Kuhn and Roth[7] the product proved to be a hexaacetate.

Anal. Calcd. for $C_{27}H_{37}O_7N(COCH_3)_6$: CH_3CO, 34.62. Found: CH_3CO, 36.0.

Alkaline Hydrolysis.—One gram of zygadenine was dissolved in 3.5 ml. methanol and 3.5 ml. of 2 *N* sodium hydroxide was added. After warming for twenty minutes at 55–60° in a water-bath the solution was refluxed ten minutes longer, and then permitted to cool slowly to room temperature. The alkalinity was reduced by the addition of 2.3 ml. of 2 *N* hydrochloric acid and 5–6 ml. of the alcohol was removed at 30°. The mixture was diluted with water and extracted with chloroform. The chloroform extract was washed with water, dried over anhydrous sodium sulfate and distilled at 30° *in vacuo*. The residue was a chloroform addition product. After drying in a vacuum desiccator at room temperature the product lost 15.83% at 100° *in vacuo* leaving 0.97 g. of recovered base (Calcd. for $C_{27}H_{43}O_7N \cdot CHCl_3$: $CHCl_3$, 19.5%). This material proved to be a mixture. It gave the same color test as zygadenine with concentrated sulfuric acid, and showed approximately the same specific rotation. However when recrystallized from acetone needles separated which showed the indefinite melting point of a mixture. These decomposed at 155–165°, first showing signs and sintering at 140°. Furthermore the solvent could not be removed completely without some decomposition. The alkaline layer freed from chloroform was acidified with phosphoric acid and distilled with steam. Titration of the distillate required 3.1 ml. of 0.05 *N* alkali, which indicated that this alkaloid is not an ester since the liberation of one molecule of volatile organic acid would have required 34.9 ml. of 0.05 *N* alkali for neutralization. Further investigation proved the entire absence of non-volatile acid.

Acid Hydrolysis.—The alkaloid (0.60 g.) was dissolved in 13.3 ml. of 2.08 *N* hydrochloric acid in excess of the amount used for neutralization and diluted to 50 ml. This 2% solution was heated at 102° for one hour. On standing nothing separated. It was extracted with chloroform, the aqueous layer made alkaline and exhausted with chloroform. The chloroform extract was washed with water, dried over anhydrous sodium sulfate and the solvent removed. There was recovered 0.47 g. of deeply colored oil from which crystalline material was not obtained.

The alkaline aqueous layer was exactly neutralized to methyl red and evaporated to dryness at 30°. The organic matter was separated from sodium chloride by leaching with alcohol. This alcoholic solution showed no reducing action with Fehling solution and gave no phenylosazone.

Summary

Zygadenine has the formula $C_{27}H_{43}O_7N$. It forms a normal sulfate $(C_{27}H_{43}O_7N)_2 \cdot H_2SO_4$ which decomposes at 237–242°. The very soluble hydrochloride $C_{27}H_{43}O_7N \cdot HCl$ decomposes at 231–234°. It forms a hexaacetate that melts with decomposition at 265–270°. The gold double salt $C_{27}H_{43}O_7N \cdot HCl \cdot AuCl_3 \cdot H_2O$ is highly crystalline but has no definite decomposition point.

Zygadenine appears to be a new alkamine, closely related to already known alkamines cevine and germine but containing one less oxygen atom.

KALAMAZOO, MICHIGAN RECEIVED JANUARY 7, 1949

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(7) Kuhn and Roth, *Ber.*, **66**, 1274 (1933).