of the $-CH_2CO-$group.[13,14] The spectral data and the elemental analysis of fraction B indicate that the compound is 1,5-diphenyl-3-methylpentane-1,5-dione. *Anal.* Calcd. for $C_{18}H_{18}O_2$: C, 81.17; H, 6.81. Found: C, 81.33; H, 6.88. The diketone gave a dioxime, m.p. 177–178° (from an ethanol–water mixture). *Anal.* Calcd. for $C_{18}H_{20}N_2O_2$: C, 72.95; H, 6.80; N, 9.46. Found: C, 73.06; H, 6.89; N, 9.42.

(13) N. B. Colthup, *J. Opt. Soc. Amer.*, **40**, 397 (1950).
(14) S. J. Francis, *J. Chem. Phys.*, **19**, 942 (1951).

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF WASHINGTON]

Azulene. IX. Synthesis of Some Derivatives of 1-Azulenethiol and 1,3-Azulenedithiol[1-3]

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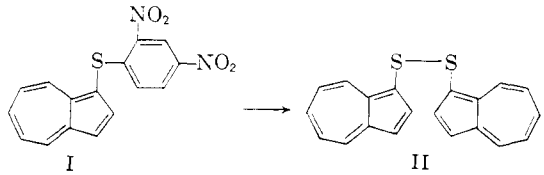
Azulene has been found to react with 2,4-dinitrobenzenesulfenyl chloride and with thiocyanogen in the absence of catalysts. From the substitution products obtained a number of new derivatives of 1-azulenethiol and 1,3-azulenedithiol have been synthesized. Efforts to prepare the unsubstituted thiol compounds were unsuccessful.

The literature on the chemistry of azulenes includes only one report of the attachment of a sulfur atom to the aromatic nucleus. Treibs and Schroth[4] accomplished the sulfonation of guaiazulene with dioxane–sulfur trioxide and conversion of the sulfonic acid to the corresponding acid chloride and amide. The present investigation was directed initially toward the synthesis of 1-azulenethiol and 1,3-azulenedithiol. The isolation of these compounds has not been achieved but a number of derivatives of them have been prepared.

Buess and Kharasch[5] have described the preparation of thiophenols through electrophilic substitution with 2,4-dinitrobenzenesulfenyl chloride and cleavage of the aryl 2,4-dinitrophenyl sulfide with alkali. Treatment of azulene with the sulfenyl chloride in the presence of aluminum chloride or stannic chloride gave only a low yield of the desired product I. It was apparent that the complexes formed by azulene and the Lewis acid catalysts were quite insoluble in the reaction mixture. Kharasch and co-workers[5,6] had found that activated benzene systems (dialkylanilines) required no catalyst. When the sulfenyl chloride and azulene were brought together in a dichloromethane solution a spontaneous reaction began at once and an 82% yield of 2,4-dinitrophenyl azulyl sulfide (I) resulted. Treatment of this product with methanolic alkali afforded a low yield of di-1-azulyl disulfide (II) but none of the desired 1-azulenethiol. All attempts to prepare the latter from I failed.



I II

Reductive cleavage of II represented another possible route to 1-azulenethiol and the preparation of the former from azulene and sulfur monochloride was tried. The reaction was run with excess azulene to minimize polymeric disulfide formation, but only gummy mixtures were obtained. The single pure substance isolated after treatment of the gummy product with lithium aluminum hydride was not a thiol and was not identified. A 1-substituted azulene would not be expected to form polymeric products. Reaction of 1-nitroazulene with sulfur monochloride and aluminum chloride gave a dark red substance thought to be bis-(3-nitroazulyl) disulfide which was not obtained analytically pure, a smaller quantity of a product identical with 1-nitro-3-chloroazulene prepared by the chlorination of 1-nitroazulene with N-chlorosuccinimide, and a small amount of a rather unexpected product, 1,3-dinitroazulene. The difficulty in the purification of the main product led to the examination of other reactions for the introduction of a sulfur atom onto the azulene nucleus.

The use of thiocyanogen to form aromatic thiocyanates has been described by Wood and Fieser,[7] and by Brewster and Schroeder.[8] Reaction of equimolar amounts of azulene and thiocyanogen at 0–5° gave a 93.5% net yield of 1-thiocyanoazulene (III). The same proportions at room temperature gave a mixture of III (45%) and 43% of 1,3-dithiocyanoazulene (IV). When two equivalents of thiocyanogen were used at 0–5°, 23% of III and 77% of IV were formed. The infrared spectrum of III showed a sharp peak at 4.65 μ. Miller,[9] reports a value of 4.63 μ for the thiocyano group.

III IV

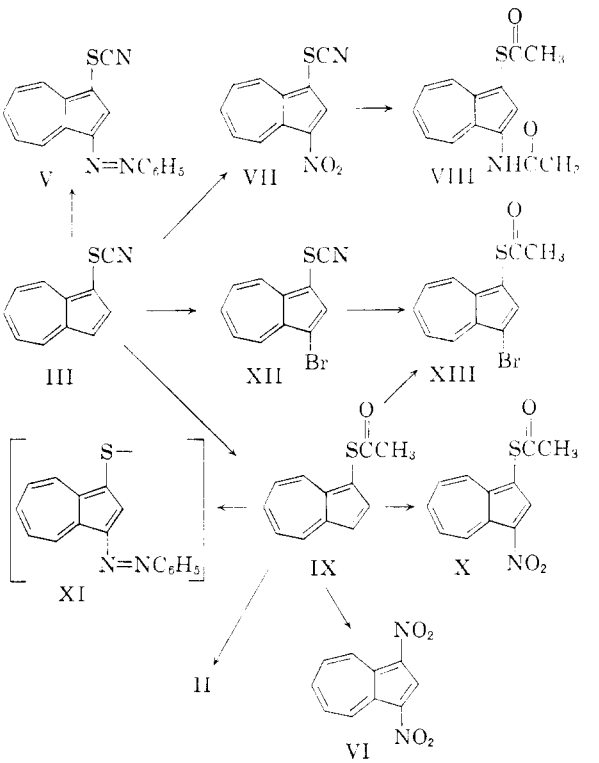
(1) From the Ph.D. thesis of Richard N. McDonald, University of Washington, 1957.
(2) Support for a part of this work by contracts DA-04-200-ORD-235 and DA-04-200-ORD-601 with the Office of Ordnance Research, U. S. Army, is gratefully acknowledged.
(3) Presented in part at the 134th Meeting of the American Chemical Society, Chicago, Ill., September, 1958.
(4) W. Treibs and W. Schroth, *Ann.*, **586**, 202 (1954).
(5) C. M. Buess and N. Kharasch, THIS JOURNAL, **72**, 3529 (1950).
(6) N. Kharasch, C. M. Buess and W. King, *ibid.*, **75**, 6035 (1953); N. Kharasch and S. J. Assony, *ibid.*, **75**, 1087 (1953).
(7) J. L. Wood and L. F. Fieser, *ibid.*, **63**, 2323 (1941).
(8) R. Q. Brewster and W. Schroeder, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 574.
(9) F. A. Miller in "Organic Chemistry. An Advanced Treatise," edited by H. Gilman, Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1953, p. 145.

Compound III was found to couple with benzenediazonium chloride under buffered (sodium acetate) conditions to give 94% net yield of V but, for unknown reasons, with an 80% recovery of III. Under more basic conditions none of the product was formed. Treatment of III with tetranitromethane and pyridine gave no reaction. Reaction with nitric acid in acetic anhydride gave starting material and a little 1,3-dinitroazulene (VI).[10] Nitration was successfully accomplished with cupric nitrate and acetic anhydride[11] to give 1-thiocyano-3-nitroazulene (VII) in 50% yield along with a small amount of 1,3-dinitroazulene. Reaction of VII with zinc, acetic acid and acetic anhydride gave the expected color change (to a blue solution which yielded green crystals) but the shift in the principal maximum of the visible absorption (to 608 mμ) did not agree with that calculated (*ca.* 595 mμ)[12] for the anticipated product, 1-thiocyano-3-acetamidoazulene. Also, the infrared spectrum did not show absorption at 4.65 μ (see above) but had a slightly split band at 5.97 μ which was suggestive of the presence of two different carbonyl groups. The elementary analysis and the above data were consistent with the formulation of the product as S-acetyl-3-acetamido-1-azulenethiol (VIII). In view of this interesting transformation of the thiocyano group,[13] the reaction was repeated with 1-thiocyanoazulene (III) under the same conditions (room temperature). No reaction occurred and the starting material was recovered. From the same reaction carried out at reflux temperature for one hour were obtained a 23% yield of the disulfide II and a 68% yield of a blue oil which was almost certainly S-acetyl-1-azulenethiol (IX). The latter product showed strong carbonyl absorption at 5.87 μ and no absorption for a thiocyano group. It formed a trinitrobenzeneate which was crystalline but difficult to purify. On chromatography over activated, basic alumina the compound was converted to II in good yield (65% from III). Since the product residue from the reaction on III was chromatographed over alumina (acid-washed), it is possible that the disulfide obtained at that point was formed from IX on the column. Indeed, when the initial reaction product was chromatographed on basic alumina, only the disulfide was obtained. All attempts to obtain 1-azulenethiol from acid or base-catalyzed hydrolysis of IX were unsuccessful.

The nitration of IX with a one molar excess of cupric nitrate in acetic anhydride gave 1,3-dinitroazulene (VI) as the only identifiable product. When equivalent amounts of cupric nitrate and IX were used, S-acetyl-3-nitro-1-azulenethiol (X) was formed but in only 11% yield. Reaction of IX with the mild nitrating mixture of tetranitromethane and pyridine also gave a low (13%) yield of X. These poor yields were attributed to side reactions involving the sensitive thio ester group. Resinous material insoluble in dichloromethane was formed in each case.

Treatment of IX with benzenediazonium chloride gave 39% of a product which showed no carbonyl absorption and thus could not be S-acetyl-3-phenylazo-1-azulenethiol. The previously noted conversion of IX to II suggested that the substance might be bis-(3-phenylazoazulyl) disulfide (XI) and the elementary analysis and spectra were in agreement with this formulation. An attempt to prepare this compound by the bis coupling of II with benzenediazonium chloride failed. Some reaction occurred but no product corresponding to XI could be isolated.

When IX was treated with N-bromosuccinimide, two products were obtained, a small amount of II and a blue oil (50%) thought to be the expected S-acetyl-3-bromo-1-azulenethiol (XIII). Neither



a picrate nor a trinitrobenzene derivative of the oil could be prepared and the substance was so unstable it could not be obtained analytically pure. Evidence for its identity was provided by the visible absorption spectrum which showed the principal maximum at 595 mμ. This is in good agreement with the value calculated (597 mμ) from the individual effects (+25 mμ for bromine and −8 mμ for S-acetylthiol) of the two groups. Further indication that the oil was XIII was obtained from its synthesis by another route. Bromination of

(10) Side reactions wherein groups other than hydrogen on the 1-position of azulene are displaced during electrophilic substitution reactions are quite common; *cf.* below and ref. 11.

(11) A. G. Anderson, Jr., J. A. Nelson and J. J. Tazuma, THIS JOURNAL, **75**, 4980 (1953).

(12) The thiocyano group causes a hypsochromic shift of 28 mμ and the acetamido group a bathochromic shift of about 45 mμ. The difference of these added to the $\lambda_{max.}$ for azulene (580 mμ) gives a good approximation of the $\lambda_{max.}$ to be expected; *cf.* A. G. Anderson, Jr., R. Scotoni, Jr., E. J. Cowles and C. G. Fritz, *J. Org. Chem.*, **22**, 1193 (1957), and references cited therein.

(13) Attempts to cleave III with acid or base were unsuccessful, although the latter gave a green semi-solid which may have contained some of the disulfide II. J. Ross, THIS JOURNAL, **56**, 727 (1934), found that organic thiocyanates normally gave disulfides on treatment with base. D. J. Gale, Ph.D. thesis, University of Washington, 1957, treated III with sodium amide and obtained II in 70% yield.

III with N-bromosuccinimide afforded 1-thiocyano-3-bromoazulene (XII) in 69% yield along with small amounts of IV and 1,3-dibromoazulene. Application of the reductive cleavage and acetylation procedure (see above) to XII gave a product identical with that obtained from the bromination of IX.

The reaction of chlorine with suspensions of thiol esters in water has been reported to give sulfonyl chlorides.[14] Treatment of XIII in this manner gave decomposition with the formation of tars. Since this procedure has also been used with organic thiocyanates,[15] it was tried with XII. Only an insoluble tar and a little starting material were obtained.

The conversion of thiocyanates to thiophenols by reduction with lithium aluminum hydride is known.[16] Application of this reaction to IV apparently gave reduction of the azulene ring and no identifiable products were isolated. Analogous results were obtained with lithium borohydride and sodium borohydride.

The assignment of the structures of the new azulene derivatives described above as 1- and 1,3-substitution products was based on the following considerations. Non-catalyzed substitution by 2,4-dinitrobenzenesulfenyl chloride almost certainly involves either ionization to form the sulfenium ion species or direct nucleophilic displacement of a chloride ion by the azulene. Either process represents electrophilic substitution on azulene and will occur at the 1-position.[11] Since the same azulyl disulfide is formed from both the 2,4-dinitrophenyl azulyl sulfide and the thiocyanoazulene, the latter must have the substituent in the 1-position. This is also in accord with the known behavior of thiocyanogen as a pseudo halogen toward other aromatic systems.[7] This is pictured as involving an electrophilic-like substitution process with the abstraction of the proton by the remaining portion of the thiocyanogen to form thiocyanic acid. Finally, both the individual and additive effects of the groups on the principal maximum in the visible spectrum correspond to those expected for 1- and 1,3-substitution products. It is very unlikely that this correlation would hold for any other possible structures.

Experimental[17]

2,4-Dinitrophenyl Azulyl Sulfide (I).—To a solution of 44 mg. (0.372 mmole) of azulene in 5 ml. of dry 1,2-dichloroethane was added 87.3 mg. (0.372 mmole) of 2,4-dinitrobenzenesulfenyl chloride. The color of the solution changed to red almost at once. The reaction mixture was refluxed for 15 min. and the solvent then removed. The residue was dissolved in a little dichloromethane–petroleum ether (1:9) and chromatographed on a column of activated, acid-washed alumina wet with the same solvent. The single brown band which developed was eluted with 3:2 dichloromethane–petroleum ether as a deep red solution. Removal of the solvent (air stream) and recrystallization of the residue from dichloromethane–petroleum ether gave 92.2 mg. (82%) of deep maroon plates, m.p. 158–159°. A chloroform solution showed λ_{max} in mμ (D_{max}) at 238 (0.68) 241 (0.69), 290 (1.32), 343 (0.50), 358 (0.46), 548 (0.84), shoulder at 590 (0.70) and a shoulder at 658 (0.25).

Anal. Calcd. for $C_{16}H_{10}O_4N_2S$: C, 58.89; H, 3.09. Found: C, 58.96; H, 3.11.

Reaction of 1-Nitroazulene and Sulfur Monochloride.—To a stirred solution of 100 mg. (0.58 mmole) of 1-nitroazulene in 15 ml. of dry dichloromethane was added 79 mg. (0.58 mmole) of sulfur monochloride in 3 ml. of dichloromethane and 60 mg. of aluminum chloride. After a few minutes the solution darkened and some solid material separated. At the end of 1 hour the mixture was poured into water, the whole extracted with dichloromethane and the combined organic solutions washed with water and dried over sodium sulfate. The residue remaining after removal of the solvent (air stream) was chromatographed on activated, acid-washed alumina with dichloromethane as the solvent. Small red and orange fractions were collected separately. From the former was obtained, after recrystallization from dichloromethane–petroleum ether, 5.5 mg. (5%) of 1-nitro-3-chloroazulene as reddish-brown rods, m.p. 185–186°, which were identical (ultraviolet and visible spectrum, m.p. and m.m.p.) with the product from the reaction (below) of 1-nitroazulene with N-chlorosuccinimide.

The ultraviolet and visible spectra of the orange eluate were identical with those of 1,3-dinitroazulene.[11] Further elution of the column with chloroform–dichloromethane (1:5) removed a larger red fraction. Recrystallization of the residue (64 mg.) from this from dichloromethane–*n*-hexane gave 51.3 mg. (43%) of a deep red solid, m.p. 190–196° dec. Two further recrystallizations afforded deep maroon plates, m.p. 200° dec. A chloroform solution showed λ_{max} in mμ (D max) at 239 (0.97), 310 (1.24), 397 (0.38), 465 (1.92) and a shoulder at 510 (1.20). Elementary analysis did not give results in satisfactory agreement with those calculated for bis-(3-nitroazulyl) sulfide.

1-Nitro-3-chloroazulene.—Twenty-three and two-tenths mg. (0.134 mmole) of 1-nitroazulene was treated with a large excess of N-chlorosuccinimide in dry benzene under reflux for 42 hr. The colorless crystals which formed when the mixture was cooled were separated and the solvent removed (air stream) from the filtrate. A solution of the residue in a minimum volume of dichloromethane was placed on activated, acid-washed alumina column wet with petroleum ether. A red-brown band was eluted with dichloromethane and removal of the solvent (air stream) from this fraction followed by recrystallization of the residue from *n*-hexane gave 4 mg. (15%) of reddish-brown rods, m.p. 184–186°. A cyclohexane solution showed λ_{max} in mμ (D_{max}) at 276 (1.67), 312 (1.65), 320 (1.65), 393 (0.91), 560 (1.10), shoulder at 603 (0.9) and 666 (0.36).

Anal. Calcd. for $C_{10}H_6O_2NCl$: C, 57.90; H, 2.92. Found: C, 57.06; H, 2.68.

1-Thiocyanoazulene (III).—To a stirred solution of 200 mg. (1.56 mmoles) of azulene in 10 ml. of dry tetrachloromethane at 0–5° was added a solution of thiocyanogen. (The latter was prepared from 555 mg. (1.72 mmoles) of lead thiocyanate in 10 ml. of dry tetrachloromethane by the addition of a solution of bromine in tetrachloromethane until the bromine color persisted, at which point just enough lead thiocyanate was added to take up the remaining bromine.) The reaction mixture, which had turned purple during the addition of thiocyanogen, was stirred at 0° for one hour and the solvent then removed (air stream). A solution of the residue in a small volume of dichloromethane was placed on an activated, acid-washed alumina column wet with petroleum ether. Elution with petroleum ether gave a solution from which was recovered 90 mg. of azulene. A purple band which developed was washed from the column with dichloromethane–petroleum ether (1:4) and, after removal of the solvent, afforded 152 mg. (93.5% net yield) of 1-thiocyanoazulene as purple needles, m.p. 76.5–77.5°. A solution in *n*-hexane showed λ_{max} in mμ (D_{max}) at 230 (1.25), 285 (1.69), 332 (0.31), 340 (0.33), 356 (0.31), 552 (1.66), 567 (1.65), 594 (1.57) and 653 (0.68). The visible absorption maximum of a chloroform solution was at 551 mμ.

Anal. Calcd. for $C_{11}H_7NS$: C, 71.32; H, 3.81. Found: C, 71.21; H, 3.56.

(14) I. B. Douglass and T. B. Johnson, THIS JOURNAL, **60**, 1486 (1938); R. C. Thomas and L. J. Ree, *ibid.*, **78**, 6150 (1956).

(15) T. B. Johnson and I. B. Douglass, *ibid.*, **61**, 2549 (1939).

(16) J. Strating and H. J. Backer, *Rec. trav. chim.*, **69**, 638 (1950).

(17) Melting points were taken on a calibrated Fisher–Johns apparatus and are uncorrected. Visible and ultraviolet absorption spectra were recorded with a Cary model 115 spectrophotometer. Infrared spectra were recorded by a Perkin–Elmer model 21 spectrophotometer. Elemental analyses were performed by Mr. B. Nist and Mr. C. H. Ludwig.

1,3-Dithiocyanoazulene (IV). Method A.—A stirred solution of 300 mg. (2.34 mmoles) of azulene in 15 ml. of dry tetrachloromethane was treated with a solution of thiocyanogen (prepared from 833 mg. (2.58 mmoles) of lead thiocyanate as described above) at room temperature. After one hour the solvent was removed and a solution of the residue in the minimum volume of dichloromethane placed on an activated, acid-washed alumina column wet with petroleum ether. A purple band was eluted with dichloromethane–petroleum ether (1:4) and recrystallization of the residue from this from dichloromethane–*n*-hexane gave 194 mg. (45%) of 1-thiocyanoazulene as purple needles, m.p. 76–77°.

Further elution with dichloromethane removed a rose band. Recrystallization of the residue from this eluate fraction gave 250 mg. (43%) of 1,3-dithiocyanoazulene as rose-red needles, m.p. 142–144°. A sample recrystallized from the same solvent melted at 149–150°. A chloroform solution showed λ_{max} in mμ (D_{max}) at 239 (0.64), 294 (1.45), 334 (0.23), 358 (0.27) and a broad peak at 535 (1.52).

Anal. Calcd. for $C_{12}H_6N_2S_2$: C, 59.48; H, 2.49. Found: C, 59.25; H, 2.37.

Method B.—To a stirred solution of 400 mg. (3.13 mmoles of azulene in 20 ml. of dry carbon tetrachloride at 0° was added a solution of thiocyanogen (prepared from 1.1 g. (3.42 mmoles) of lead thiocyanate as described above). A second equal portion of thiocyanogen solution was added after one hour and the solution color changed from purple to red. After another hour at 0° with stirring, the mixture was taken to dryness (air stream) and a solution of the residue in the minimum volume of dichloromethane placed on a column of activated, acid-washed alumina wet with petroleum ether. Elution with dichloromethane–petroleum ether (1:4) gave a purple fraction from which was obtained 133 mg. (23%) of 1-thiocyanoazulene as purple needles, m.p. 76–77°. A rose colored band was eluted with dichloromethane and the residue from this, after recrystallization from *n*-hexane–dichloromethane, yielded 583 mg. (77%) of 1,3-dithiocyanoazulene as rose-red needles, m.p. 142–144°, identical (m.p., m.m.p., infrared spectrum) with the material described above (method A).

1-Thiocyano-3-phenylazoazulene (V).—To a solution of 50 mg. (0.27 mmole) of 1-thiocyanoazulene and *ca.* 0.2 g. of sodium acetate in 5 ml. of 95% ethanol at 0° was added a solution of benzenediazonium chloride (prepared at −5–0° from 80 mg. (0.615 mmole) of pyridinium chloride, 2 ml. of water, 0.5 ml. of concentrated hydrochloric acid and 40 mg. (0.580 mmole) of sodium nitrite. Needles precipitated immediately and the mixture was allowed to warm to room temperature. After 20 hours the mixture was poured into water and the whole extracted with dichloromethane. The combined extracts were washed with dilute potassium hydroxide and water and then dried over sodium sulfate. The solvent was evaporated with an air stream and the residue, dissolved in the minimum volume of dichloromethane, chromatographed on a column of activated, acid-washed alumina wet with petroleum ether. A 1:4 dichloromethane-petroleum ether solvent eluted 41 mg. of 1-thiocyanoazulene. Further elution with 3:4 dichloromethane–petroleum ether removed a red band as a yellow solution. The last traces of this material were washed off the column with 1:1 and then 4:3 dichloromethane–petroleum ether. From the combined fractions were obtained 13.2 mg. (94% net yield) of product which crystallized from *n*-hexane–dichloromethane as clusters of green needles, m.p. 138–140° dec. The analytical sample melted at 139–140° dec. A cyclohexane solution showed λ_{max} in mμ (D_{max}) at 232 (1.85), 283 (1.48) and 403 (1.44). The visible region had a shoulder at 580 (1.13) but no distinct maxima.

Anal. Calcd. for $C_{17}H_{11}NsS$: C, 70.56; H, 3.83. Found: C, 70.32; H, 3.71.

1-Thiocyano-3-nitroazulene (VII).—To a stirred solution of 101 mg. (0.55 mmole) of 1-thiocyanoazulene in 5 ml. of acetic anhydride cooled by a Dry Ice–acetone-bath was added a suspension of 140 mg. (0.58 mmole) of cupric nitrate in 5 ml. of acetic anhydride. The cooling bath was removed after 5 min. and stirring was continued for 90 min. The mixture was then poured into water and extracted with dichloromethane. The combined extracts were washed with dilute potassium hydroxide, water, and dried over sodium sulfate. The solvent was removed (air stream), the residue dissolved in the minimum volume of dichloromethane, and the solution placed on a column of activated, acid-washed alumina wet with petroleum ether. Dichloromethane eluted a red-orange band and then a small orange band. The ultraviolet and visible spectra of the latter were identical to those of 1,3-dinitroazulene.[11] The solvent was removed (dry air stream) from the red-orange fraction and the residue recrystallized from dichloromethane–petroleum ether to give 62 mg. (50%) of orange-red needles, m.p. 174–176°. The analytical sample melted at 176–177°. A chloroform solution showed λ_{max} in mμ (D_{max}) at 240 (0.81), 282 (1.40) 312 (1.07) and 386 (0.67). The visible spectrum had a single, rather broad maxima at 500 (1.82).

Anal. Calcd. for $C_{11}H_6O_2N_2S$: C, 57.38; H, 2.63. Found: C, 57.45; H, 2.48.

S-Acetyl-3-acetamido-1-azulenethiol (VIII).—To a stirred solution of 51 mg. (0.222 mmole) of 1-thiocyano-3-nitroazulene and 0.4 g. of sodium acetate in 10 ml. of glacial acetic acid and 10 ml. of acetic anhydride at room temperature was added 1.0 g. of zinc dust over a period of 5 min. The color of the solution changed to blue as the zinc was added. After one hour the suspension was poured into water and the whole extracted with dichloromethane. The combined green-blue extracts were washed with dilute potassium hydroxide and water, and then dried over sodium sulfate. The solvent was removed (air stream) and a solution of the residue in the minimum volume of dichloromethane was placed on a column of activated, acid-washed alumina wet with dichloromethane. Blue and green bands developed on the column and the blue one was eluted with chloroform. Removal of the solvent (dry air stream) from this eluate and recrystallization of the residue from *n*-hexane–dichloromethane afforded 36 mg. (63%) of product as green needles, m.p. 191–192°. The analytical sample melted at 192–193°. A chloroform solution showed λ_{max} in mμ (D_{max}) at 242 (0.87), 297 (1.36), 368 (0.25), a shoulder at 386 (0.19) and a broad peak at 608 (1.15).

Anal. Calcd. for $C_{14}H_{13}O_2NS$: C, 64.84; H, 5.05. Found: C, 64.74; H, 4.98.

S-Acetyl-1-azulenethiol (IX).—To a stirred solution of 398 mg. (2.15 mmoles) of 1-thiocyanoazulene and 1.0 g. of sodium acetate in 15 ml. of glacial acetic acid and 15 ml. of acetic anhydride was added 2.0 g. of zinc dust. The mixture was heated under reflux with stirring for 90 min., then poured into water and the whole extracted with dichloromethane. The combined extracts were washed with dilute potassium hydroxide and water, and then dried over sodium sulfate. The solvent was removed (air stream) and a solution of the residue in the minimum volume of petroleum ether placed on an activated, acid-washed alumina column wet with petroleum ether. A green band was eluted as a blue-green solution with 1:9 dichloromethane–petroleum ether and yielded 79 mg. (23%) of di-1-azulyl disulfide (II) as dark green needles, m.p. 116–118°, identical (m.m.p., ultraviolet, visible and infrared spectra) with an authentic sample (see below).

Further elution with 3:7 dichloromethane–petroleum ether removed a purple-blue band and evaporation (dry air stream) of the solvent from this fraction left 293 mg. (68% of product as a blue oil which could not be crystallized. A *n*-hexane solution showed λ_{max} in mμ (D_{max}) at 228 (1.47), 287 (1.35), 343 (0.20), 360 (0.19), 572 (1.26), 591 (1.17), 618 (1.11), 658 (0.51) and 682 (0.39). The infrared spectrum showed a strong band at 5.87 μ and no absorption characteristic of the thiocyano group. The oil was not obtained sufficiently pure to give satisfactory elementary analyses.

A solution of 39 mg. of the blue oil and 41 mg. of trinitrobenzene (prepared as a saturated solution in ethanol at 0°) in ethanol was heated to boiling and then cooled in an ice-bath. Dark brown needles separated which melted at 94.5–95.5° before and after recrystallization from ethanol.

Anal. Calcd. for $C_{18}H_{13}O_7N_3S$: C, 51.43; H, 3.12. Found: C, 52.05; H, 2.98.

Di-1-azulyl Disulfide (II). A. From 1-Thiocyanoazulene (III).—As described above for the preparation of IX, 195 mg. (0.05 mmole) of 1-thiocyanoazulene was treated with zinc, acetic acid and acetic anhydride. The desired product (IX), separated by chromatography on activated, acid-washed alumina, was placed on a column of activated, basic alumina wet with petroleum ether. The purple band changed rapidly to green and was eluted as a green solution with 1:95 dichloromethane–petroleum ether. Removal of the solvent (air stream) and recrystallization of the residue from

n-hexane–dichloromethane gave 109 mg. (65%) of II as clusters of dark green needles, m.p. 114–116°. The analytical sample melted at 118–119°. A cyclohexane solution showed λ_{max} in mμ (D_{max}) at 234 (1.48), 282 (2.05), 385 (0.44), 575 (1.83), 623 (1.56) and 690 (0.56).

Anal. Calcd. for $C_{20}H_{14}S_2$: C, 75.45; H, 4.40. Found: C, 75.63; H, 4.52.

B. From 2,4-Dinitrophenyl Azulyl Sulfide (I).—A deep red solution of 96.8 mg. (0.297 mmole) of 2,4-dinitrophenyl azulyl sulfide and 390 mg. of potassium hydroxide in 15 ml. of ethanol was heated under reflux for 30 min. The volume was then reduced to 8 ml., a second portion of *ca.* 390 mg. (three pellets) of potassium hydroxide added, and refluxing resumed for 30 min., during which time some solid separated. The cooled mixture was poured into dilute potassium hydroxide and the whole was extracted several times with dichloromethane. The combined red extracts yielded unreacted starting material (I) as shown by comparison of the absorption spectra, m.p. and m.m.p. with those of an authentic sample.

The aqueous layer became turbid when acidified with hydrochloric acid and was then extracted with dichloromethane. The combined green extracts were dried over sodium sulfate and evaporated (air stream) to dryness. The green residue (58 mg.) was chromatographed on a column of activated, acid-washed alumina with 1:4 dichloromethane–petroleum ether. A blue-green solution eluted at once (a small, light green band was then removed with dichloromethane but was not investigated further), was concentrated to a small volume (air stream) and rechromatographed on activated, basic alumina. Dichloromethane–petroleum ether (1:9) eluted a brown-green band (a small, light green band remained strongly adsorbed) to give a blue-green solution. Evaporation of the solvent left a green residue which crystallized when triturated with a few drops of petroleum ether and gave 5.6 mg. of green needles, m.p. 109–112°. The visible, ultraviolet and infrared spectra of this material were identical with those of the product obtained in A.

1,3-Dinitroazulene (VI) from S-Acetyl-1-azulenethiol (IX).—To a stirred solution of 124 mg. (0.61 mmole) of S-acetyl-1-azulenethiol in 5 ml. of acetic anhydride cooled by a Dry Ice–acetone-bath was added a suspension of 0.18 g. (0.75 mmole) of cupric nitrate trihydrate in 7 ml. of acetic anhydride. The cooling bath was removed after 5 min. and mixture stirred for two hours. The color of the solution changed to a dark green a few minutes after the bath was removed and then to red in about 30 min. The mixture was then poured into water and extracted with dichloromethane. An emulsion formed which was not affected by the addition of potassium hydroxide or sodium chloride but was broken by an excess of concentrated hydrochloric acid. The combined extracts were washed with dilute potassium hydroxide (some red color went into the aqueous layer) and dried over sodium sulfate. Removal of the solvent (air stream) from the orange solution and chromatography of the residue on activated, acid-washed alumina with dichloromethane gave an orange band. This was eluted and yielded 34 mg. (25.6 %) of orange needles, m.p. 262–263° which were identical (m.m.p., infrared spectrum) with an authentic sample.

S-Acetyl-3-nitro-1-azulenethiol (X). Method A.—To a solution of 43 mg. (0.213 mmole) of S-acetyl-1-azulenethiol in 3 ml. of dry pyridine was added 0.5 ml. of tetranitromethane in 1.5 ml. of absolute ethanol. After 5 min. the color of the solution had changed from blue to red. After another 10 minutes the mixture was poured into dilute hydrochloric acid and the whole extracted with dichloromethane, washed with water, and dried over sodium sulfate. The solvent was removed (air stream) from the intense red solution and a solution of the residue in a mixture of dichloromethane and chloroform was chromatographed on activated, acid-washed alumina. Elution with chloroform gave a red eluate fraction and the residue from this was rechromatographed with dichloromethane as the eluent. The orange band which developed eluted as a red-orange solution and evaporation (dry air stream) of the solvent left a red solid. This recrystallized from *n*-hexane–dichloromethane as red needles, m.p. 184.5–187° dec., and amounted to 7 mg. (13%). The analytical sample, after sublimation at 130° and 1 mm., melted at 185–186.5° dec. A chloroform solution showed λ_{max} in mμ (D_{max}) at 240 (1.18), 284 (1.57), 315 (1.27) 403 (0.78) and 512 (1.31).

Anal. Calcd. for $C_{12}H_9O_3NS$: C, 58.28; H, 3.67. Found: C, 58.15; H, 3.96.

Method B.—To a stirred solution of 45 mg. (0.223 mmole) of S-acetyl-1-azulenethiol in 5 ml. of acetic anhydride cooled by a Dry Ice–acetone bath was added a suspension of 27 mg. (0.112 mmole) of cupric nitrate trihydrate in 3 ml. of acetic anhydride. The cooling bath was removed after 10 minutes and the mixture stirred at room temperature for 90 minutes during which period the color changed to blue-green and finally to green. The mixture was poured into water, the whole extracted with dichloromethane, and the combined extracts washed with dilute potassium hydroxide and dried over sodium sulfate. The solvent was removed (air stream) from the red solution and the residue chromatographed on a column of activated, acid-washed alumina wet with dichloromethane. Elution with the same solvent gave a red-orange solution which yielded 6 mg. (11%) of red needles, m.p. 178–183.5° dec., identical (ultraviolet, visible and infrared spectra) with the product obtained in A.

Bis-(3-phenylazoazulyl) Disulfide (XI).—A solution of benzenediazonium chloride (prepared by the combination 33 mg. (0.254 mmole) of anilinium chloride, 2 ml. of water, 0.5 ml. of concentrated hydrochloric acid and 18 mg. (0.261 mmole) of sodium nitrite cooled in an ice–salt bath, followed by the addition of a few crystals of urea) was added to a stirred solution of 45 mg. (0.222 mmole) of S-acetyl-1-azulenethiol and 0.3 g. of sodium acetate in 5 ml. of ethanol at room temperature. The solution turned an intense red at once. It was stirred for 90 minutes, then poured into water and the whole extracted with dichloromethane. The combined extracts were washed with dilute potassium hydroxide and dried over sodium sulfate. The solvent was removed (air stream) from the yellow-green solution and a solution of the residue in the minimum volume of dichloromethane placed on a column of activated, acid-washed alumina wet with petroleum ether. Elution with 3:7 dichloromethane–petroleum ether removed a small zone of di-1-azulyl disulfide and dichloromethane eluted a large red band as a yellow-red solution. Removal of the solvent (dry air stream) from the latter left a solid which, after recrystallization from *n*-hexane–dichloromethane, amounted to 23 mg. (39%) of green needles, m.p. 175–180°. Rechromatography of this with 1:1 dichloromethane–petroleum ether as the solvent gave a red-brown eluate and the residue from this crystallized from *n*-hexane–dichloromethane as blue-green needles, m.p. 188–190° dec. The analytical sample melted at 191–193° dec. A cyclohexane solution showed λ_{max} in mμ (D_{max}) at 237 (1.79), 279 (1.61), 334 (1.61), 422 (1.90), 597 (0.78), and a shoulder at 658 (0.58).

Anal. Calcd. for $C_{32}H_{22}N_4S_2$: C, 72.94; H, 4.21. Found: C, 72.92; H, 4.30.

1-Thiocyano-3-bromoazulene (XII).—A reaction mixture composed of 52 mg. (0.281 mmole) of 1-thiocyanoazulene, 5 ml. of dry dichloromethane and 55 mg. (0.306 mmole) of N-bromosuccinimide was allowed to stand at room temperature for 15 min. and the solvent then removed (air stream). A solution of the residue in the minimum volume of dichloromethane was placed on a column of activated, acid-washed alumina wet with petroleum ether. The latter solvent eluted a brilliant blue fraction and evaporation of the solvent (dry air stream) from this left 8 mg. of green needles, m.p. 73–75°, which were identical (m.m.p., visible spectrum) with an authentic sample of 1,3-dibromoazulene.

A 1:2 dichloromethane–petroleum ether solvent eluted a larger purple band as a blue solution. Removal of the solvent (air stream) and recrystallization of the residue from *n*-hexane-dichloromethane afforded 51 mg. (69%) of XII as dark green needles, m.p. 100–101° dec. The analytical sample had the same melting point. A cyclohexane solution showed λ_{max} in mμ (D_{max}) at 234 (1.04), shoulder at 290 (1.54), 294 (1.58), 299 (1.58), shoulder at 345 (0.18), 352 (0.21), 368 (0.29), 572 (1.62), shoulder at 595 (1.55), 618 (1.48) and 683 (0.61).

Anal. Calcd. for $C_{11}H_6NSBr$: C, 50.01; H, 2.29. Found: C, 49.81; H, 2.55.

Elution with dichloromethane gave a small amount of rose needles, m.p. 146–147°, identified (m.m.p., ultraviolet and visible spectra) as 1,3-dithiocyanoazulene (IV).

Reaction of S-Acetyl-1-azulenethiol with N-Bromosuccinimide.—To a solution of 30 mg. (0.149 mmole) of S-acetyl-1-azulenethiol in 5 ml. of dry dichloromethane was added 2.75 mg. (0.149 mmole) of N-bromosuccinimide at room temperature. As the mixture was swirled all of the brominating agent went into solution and the color changed to a deep

blue. After 15 minutes the solvent was removed (air stream) and a solution of the residue in the minimum volume of dichloromethane placed on a column of activated, acid-washed alumina wet with petroleum ether. The latter solvent eluted a small fraction as blue-green solution (not identified but possibly 1,3-dibromoazulene) and dichloromethane–petroleum ether (1:4) removed a small amount of material which exhibited ultraviolet and visible spectra identical with those of di-1-azulyl disulfide (II).

The principal blue band was eluted with 3:7 dichloromethane–petroleum ether and evaporation (dry air stream) of the solvent from this fraction left 21 mg. (50%) of a blue oil thought to be S-acetyl-3-bromo-1-azulenethiol (XIII). It was unstable in a nitrogen or air atmosphere and was not obtained analytically pure. Attempts to prepare trinitrobenzeneate and picrate derivatives failed. A cyclohexane solution showed λ_{max} in mμ (D_{max}) at 234 (1.30), 294 (1.75), 300 (1.76), 355 (0.24), 372 (0.31), 595 (1.37), 643 (1.19) and 713 (0.44). The infrared spectrum in the region from 5–10 mμ was quite similar to that of S-acetyl-1-azulenethiol (IX).

Reaction of 1-Thiocyano-3-bromoazulene (XII) with Zinc, Acetic Acid and Acetic Anhydride.[18]—To a stirred solution of 45 mg. (0.17 mmole) of 1-thiocyano-3-bromoazulene and 0.2 g. of sodium acetate in 5 ml. of glacial acetic acid and 5 ml. of acetic anhydride was added 0.5 g. of zinc dust. The combined extracts were washed with dilute potassium hydroxide, dried over sodium sulfate, and evaporated (air stream) to dryness. A solution of the residual oil in the minimum volume of dichloromethane was placed on a column of activated, acid-washed alumina wet with petroleum ether. The latter solvent developed faint pink and green zones and a large blue band. The pink zone disappeared and the green zone became more diffuse and finally colored most of the column. Elution with 2:1 dichloromethane–petroleum ether removed the green material which was obtained as an unstable solid (14 mg.). This substance contained sulfur and halogen and its infrared spectrum had no peak in the carbonyl region. It was possibly bis-(3-bromoazulyl)disulfide. The large blue band separated into a blue band and a smaller purple band. The former was eluted with 4:1 dichloromethane–ether and evaporation of the solvent (air stream) left 16 mg. (33%) of blue oil. A cyclohexane solution of this material had the same ultraviolet, visible and infrared spectra as that of the product from the bromination of S-acetyl-1-azulenethiol (see above).

When Florisil was used as the adsorbent the yields of products and the elution solvents were: 2 mg. of green solid (benzene–petroleum ether), 29 mg. (60%) of blue oil (4:1 benzene–dichloromethane) and 3 mg. of a purple oil of unknown identity (4:1 benzene–dichloromethane). The spectra of the blue oil were the same given above except for the presence of a maximum at 614 mμ.

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(18) The directions given are taken in part from results obtained by Mr. Lanny L. Replogle.

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The Kinetics of the Reaction of Human Erythrocyte Carbonic Anhydrase. II. The Effect of Sulfanilamide, Sodium Sulfide and Various Chelating Agents

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The kinetic behavior of human erythrocyte carbonic anhydrase was studied by a rapid recording electrometric method for the measurement of changes in hydrogen ion activity accompanying the hydration of carbon dioxide at *p*H 7.00 and 1.5° in lightly buffered solutions. The chemical inhibition of carbonic anhydrase by sulfanilamide, sodium sulfide and various chelating agents was studied at an enzyme concentration corresponding to a zinc concentration of 2.7×10^{-2} micromole per liter. Sulfanilamide and sodium sulfide were strong non-competitive inhibitors. The association constants for binding of the inhibitor by the enzyme and by the enzyme–substrate compound were identical; for sulfanilamide this constant was $1.3 \times 10^{5}\ M^{-1}$, and for sulfide (expressed as total sulfide concentration) it was $3.3 \times 10^{6}\ M^{-1}$. Methylamine, ethylenediamine, ethylenediamine tetraacetate and 1,10-phenanthroline were without inhibitory effect on short incubation with the enzyme. The inference from the sulfide inhibition data is that carbon dioxide does not bind at the zinc atom of the enzyme molecule. Sulfanilamide may bind at the metal site, but inhibition data cannot critically determine this question. From the lack of inhibition by chelating agents, it is inferred that the zinc atom is firmly bound in carbonic anhydrase by the electrons ordinarily involved in chelation of inorganic zinc. The possible role of zinc in contributing an hydroxyl group to the catalytic process is discussed.

Introduction

A previous report[2] discussed the results of a study of the basic kinetic mechanism of the human erythrocyte carbonic anhydrase catalyzed hydration of carbon dioxide. From the ionic inhibition data it was possible to determine explicitly the values of the rate constants for each step of the Michaelis–Menten mechanism. It was of interest to extend the study to possible modes of chemical inhibition beyond the previously studied non-specific ionic effects in order to elucidate the chemical aspects of the catalysis in contrast to the kinetic details.

Mann and Keilin[3] first reported the inhibition of carbonic anhydrase by sulfanilamide. Their observations were extended to a large series of compounds of the sulfonamide derivative by Miller, Dessert and Roblin,[4] culminating in the synthesis of acetazoleamide, a potent inhibitor of carbonic anhydrase.[5] Davenport[6] interpreted his data on the inhibition of carbonic anhydrase by sulfanilamide to demonstrate a competitive mechanism of inhibition. The data, however, demonstrated only the mass action principles in the inhibition but could not distinguish critically among the various conceivable mechanisms of enzyme inhibition. Since no information was available on the mechanism of inhibition, further study of this problem seemed in order.

The role of the zinc atom in the carbonic anhydrase molecule is not yet known. The data of

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