g.) of the crude reduction product, dissolved in boiling 90% ethanol (40 ml.), was treated with a hot 1% solution of digitonin in 90% ethanol (100 ml.). After being kept overnight, the precipitate was collected, washed and dried (0.8303 g.). The digitonide was heated on the steam-bath with pyridine (5 ml.) which was then removed *in vacuo*.[5] Exhaustive extraction of the residue with ether gave Δ^1-cholesten-3β-ol (II) which after several recrystallizations from methanol melted at 131–131.5°, $[\alpha]^{20}D$ +54.9°, [M]D +213.

Anal. Calcd. for $C_{27}H_{46}O$: C, 83.87; H, 11.99. Found (average): C, 83.81; H, 11.76.

The acetate was prepared by adding excess acetic anhydride to a solution of the stenol in pyridine, keeping the mixture at room temperature overnight, and precipitation of the reaction product with water. It was recrystallized several times from ether–methanol, m.p. 87°, $[\alpha]^{21}D$ +60.0°, [M]D +257, Δ_{Ac} +47.

Anal. Calcd. for $C_{29}H_{48}O_2$: C, 81.25; H, 11.29. Found: C, 81.23; H, 11.25.

The benzoate was prepared by treating a solution of the stenol in pyridine with benzoyl chloride. After standing overnight at room temperature, the mixture was diluted with methanol and the precipitate recrystallized from ether and ethyl acetate; hard needles, m.p. 141°, $[\alpha]^{25}D$ +94.5°, [M]D +464, Δ_{Be} +251.

Anal. Calcd. for $C_{34}H_{50}O_2$: C, 83.21; H, 10.27. Found: C, 82.83; H, 10.23.

Hydrogenation of Δ^1-Cholesten-3β-ol.—The stenol II was hydrogenated in ethyl acetate and at atmospheric pressure with a platinum catalyst. The 3β-cholestanol thus obtained, m.p. 141–142°, $[\alpha]^{21}D$ +28.1°, was converted into the benzoate which melted at 136° to an opalescent liquid which cleared at 154.5–155.5°, $[\alpha]^{22}D$ +22°. It gave no depression of the melting points when mixed with authentic material.

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Infrared Spectra of Some Tricyclic and Tetracyclic Lactones[1]

BY JEROME A. BERSON

RECEIVED MARCH 25, 1954

The carbonyl group of simple 5-membered lactones is characterized by absorption in the infrared at or near 5.65 μ and that of 6-membered lactones by absorption in the same region as open-chain esters, *i.e.*, at or near 5.75 μ.[2,3] In connection with other studies, we have prepared a number of tricyclic lactones derived from cyclopentadiene–maleic acid and furan–maleic acid Diels–Alder adducts. The unusual steric requirements of these substances as well as the lack of direct evidence on the size of the lactone rings[4] prompted us to examine the infrared spectra. The data settle the question of ring size and provide information on the effect of confinement of the lactone function in the relatively strained environment of the tricyclic system.

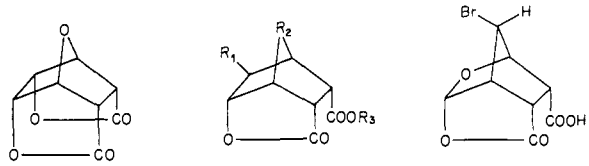
(1) Paper VI in the series "The Structure and Stereochemistry of Bicyclic Derivatives."

(2) *Cf. inter alia* (a) R. S. Rasmussen and R. R. Brattain, THIS JOURNAL, **71**, 1073 (1949); (b) R. N. Jones, P. Humphries and K. Dobriner, *ibid.*, **72**, 956 (1950).

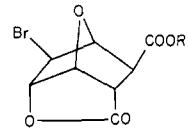
(3) It has been pointed out [R. B. Woodward, *ibid.*, **72**, 3327 (1950)] that the assignment of the region 5.48–5.50 μ to lactone absorption in the useful and informative reference work by H. M. Randall, R. G. Fowler, N. Fuson and J. R. Dangl, "Infrared Determination of Organic Structures," D. Van Nostrand Co., Inc., New York, N. Y., 1949, is based on the spectra of oxazolones and should not be taken as applying to lactones in general.

(4) *Cf.* J. A. Berson and R. Swidler, *ibid.*, **76**, 4060 (1954).

Table I gives the absorption maxima and band assignments in the carbonyl region for the substances concerned.



I IIa R_1,R_3 = H; R_2 = CH_2 III
b R_1 = Br; R_2 = CH_2; R_3 = H
c R_1 = Br; R_2 = CH_2; R_3 = CH_3
d R_1 = Br; R_2 = O; R_3 = H
e R_1 = Br; R_2 = O; R_3 = CH_3



IVa R = H
b R = CH_3

TABLE I

INFRARED MAXIMA (μ)

	Lactone	Ester or carboxyl		Lactone	Ester or carboxyl
I[a]	5.62	..	IId[c]	5.66	5.82
IIa[b]	5.64	5.82	IIe[c]	5.61	5.77
IIb[b,e]	5.66	5.73, 5.84	III[d]	5.68	5.78
IIb[b,f]	5.69	5.86	IVa[c]	5.68	5.73
IIc[b]	5.61	5.75	IVb[c]	5.61	5.77

[a] J. A. Berson and R. Swidler, THIS JOURNAL, **75**, 1721 (1953). [b] Reference 6. [c] O. Diels and K. Alder, *Ann.*, **490**, 243 (1931). [d] R. B. Woodward and H. Baer, THIS JOURNAL, **70**, 1161 (1948). [e] Anhydrous bromolactonic acid, m.p. 157°, $C_9H_9O_4Br$. [f] Bromolactonic acid hydrate, m.p. 116°, $C_9H_{11}O_5Br$.

While it might possibly be argued *a priori* that the strain introduced by the bicyclic system would have an appreciable effect on the lactone C=O stretching frequency, this does not seem to be the case. The dilactone I in which the lactone rings are necessarily 5-membered shows the normal 5-membered lactone absorption. The average position of the lactone band for all the substances of Table I is 5.65 ± 0.03 μ. Using I as a model, it is clear that all of the lactones examined here are 5-membered. The result is in agreement with general experience on the preference for γ- as against δ-lactone formation.[5]

The assignment[6] of a bromohydrin structure V to the substance $C_9H_{11}O_5Br$, m.p. 116°, obtained on bromination of *endo-cis*-3,6-endomethylene-Δ^4-tetrahydrophthalic acid, already disputed[4] on chemical grounds, is definitely invalidated by the strong lactone absorption in the infrared (Table I). This substance is therefore a crystal hydrate of the lactonic acid IIb, m.p. 157°.

It may be significant that the lactone C=O bands for the lactonic acids of the series appear at slightly longer wave lengths than those of the corresponding esters. It seems likely that this effect is associated with hydrogen bonding between the lactone and carboxyl functions.

(5) *Cf.* H. C. Brown, J. H. Brewster and H. Schechter, *ibid.*, **76**, 467 (1954).

(6) K. Alder and G. Stein, *Ann.*, **504**, 216 (1933).

Experimental

The lactones were prepared according to literature procedures and purified by recrystallization from appropriate solvents. All spectra were run in Nujol mulls. The instrument was a Perkin–Elmer model 13 recording spectrometer operated at slow chart speeds and at speed–response ratios which gave maximum resolution (estimated at 10 cm.$^{-1}$). A wave length calibration preceded each set of runs. This was achieved by operating the spectrometer on "direct mode" and recording the water vapor spectrum.

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The Preparation and Biological Properties of 4,4′-Bis-(*p*-dimethylaminophenylazo)-phenyl Disulfide[1,2]

BY R. K. BURKHARD, D. E. SETTER[3] AND R. M. GROSSMAN

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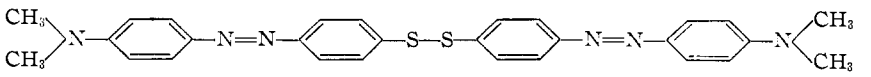
4,4′-Bis-(*p*-dimethylaminophenylazo)-phenyl disulfide (DS) was prepared for two reasons. DS



might serve as a suitable intermediate in the synthesis of azo dyes related to methyl orange but which would contain lower oxidation states of the sulfur atom than that which is found in methyl orange. The disulfide linkage in this molecule might enable it to react with mercaptan groups in a protein and hence form azo dye–protein derivatives which would contain the carcinogenic moiety found in 4-dimethylaminoazobenzene (DAB). It has been found that when carcinogenic azo dyes are fed to rats these dyes are incorporated into the liver proteins of the rat through a linkage which probably involves the dimethylamino group of the dye.[4] It might be that the feeding of DS would result in the formation of azo dye–protein derivatives in which the carcinogenic moiety found in DAB would be attached to liver proteins through a disulfide linkage. It might be that an azo dye bound to liver proteins in such a manner would also induce tumor formation.

Experimental

Preparation of 4,4′-Bis-(*p*-dimethylaminophenylazo)-phenyl Disulfide (DS).—The starting material for this synthesis was acetanilide which was converted into *p*-acetaminobenzene sulfonyl chloride according to the procedure of Smiles and Stewart.[5] The crude acid chloride was dried with an acetone–benzene mixture according to the procedure of Adams and Johnson[6] if it was to be stored for any length of time. This acid chloride was reduced to bis-(*p*-acetamino)-phenyl disulfide and hydrolyzed to 4,4′-diaminophenyl disulfide dihydrochloride according to the method of Bauer and Cymerman.[7] The tetrazotization of this diamine dihydrochloride and its subsequent coupling to dimethylaniline were accomplished in a manner similar to that used by Burawoy and Turner.[8] A suspension of 4,4′-diaminophenyl disulfide dihydrochloride (1.61 g.) in concentrated sulfuric acid (7 ml.) was prepared and cooled to 0°. This suspension was then slowly added to a cold solution of nitrosyl sulfuric acid. After tetrazotization the cold solution of the tetrazonium salt was slowly added to a cold solution of 1.21 g. of dimethylaniline in 200 ml. of 50% alcohol containing 60 g. of sodium acetate. The *p*H of the solution was intermittently measured and not allowed to go below 5.0 by the addition of more sodium acetate. The reaction mixture became very viscous and alcohol and chipped ice were added occasionally to maintain an easily stirred mixture. After the addition of the tetrazonium salt was completed the resulting mixture was then stirred for an additional 30 minutes followed by the addition of a small amount of urea. The crude product was then collected and washed several times with water to dissolve the large quantities of inorganic salts present. This procedure gave 2.1 g. (82% yield) of crude DS. The crude DS was first crystallized from a pyridine–water mixture, then from pyridine and finally from ethylene chloride to give red crystals melting at 198–199.5°. Analysis of DS for nitrogen and sulfur contents gave 15.8 and 12.0%, respectively. These data yield upon calculation a nitrogen/sulfur ratio of 1.32/1.00. The values calculated for $C_{28}H_{28}N_6S_2$ are 16.4% nitrogen, 12.5% sulfur and a nitrogen/sulfur ratio of 1.31/1.00. The absorption spectrum of DS in ethylene chloride shows a maximum at 4300 Å. with a molar extinction coefficient of 3.04×10^4.

Test for Carcinogenicity.—The procedure for testing of DS was similar to that used by the Wisconsin group wherein the dye is fed to albino rats by its incorporation into a special basal ration.[9]

Three groups of Sprague-Dawley male albino rats of weights averaging 200 g. were arranged. Each group was fed *ad libitum* for a fourteen week period one of the following rations: group I, basal ration; group II, DS ration; and group III, 3′-methyl-4-dimethylaminoazobenzene (3′-Me-DAB) ration. The DS ration and the 3′-Me-DAB ration both incorporated these dyes at 0.06% concentration by weight rather than at equal molar concentrations because it was thought that DS could be cleaved *in vivo* to yield two (*p*-dimethylaminophenylazo)-phenyl moieties. At the end of the fourteen week period the animals were anesthetized and an examination of the internal organs made.

Preparation of Blood-free Liver Homogenates.—The liver of each animal was perfused *in situ* with 0.89% saline, excised, quick frozen and then stored for a week in the frozen state. After this time the livers were homogenized and dialyzed at 0° to remove soluble colored materials and then lyophilized.

Results and Discussion

Examination of the three groups of rats showed that the livers from the rats fed the basal ration and the rats fed the DS ration could not be differentiated one from another by gross examination. The livers from the rats fed the 3′-Me-DAB ration were cirrhotic and small tumors were noted. Histological examination of the livers revealed that the livers from the DS fed rats were normal.[10] From these data it was concluded that DS is not a hepatic carcinogen for the male albino rat under the conditions of the test.

These findings brought up the question as to the

(1) Presented in part before the Biological Section of the 125th National Meeting of the American Chemical Society, Kansas City, Missouri, March 25, 1954.

(2) Supported by the National Cancer Institute, U. S. Public Health Service, Bethesda 14, Maryland.

(3) U. S. Public Health Service Predoctoral Research Fellow, 1952–1953.

(4) E. C. Miller, J. A. Miller, R. W. Sapp and G. M. Weber, *Can. Res.*, **9**, 336 (1949).

(5) S. Smiles and J. Stewart, "Organic Syntheses," Coll. Vol. I, H. Gilman and A. H. Blatt, Editors, 2nd Edition, John Wiley and Sons, Inc., New York, N. Y., 1946, pp. 8–10.

(6) R. Adams and J. R. Johnson, "Elem. Lab. Exp. in Org. Chem.," 3rd Edition, the Macmillan Co., New York, N. Y., 1945, p. 361.

(7) L. Bauer and J. Cymerman, *J. Chem. Soc.*, 3434 (1948).

(8) A. Burawoy and C. Turner, *ibid.*, 469 (1950).

(9) H. P. Rusch, C. A. Bauman, J. A. Miller and B. E. Kline, "Experimental Liver Tumors," in A. A. A. S. Res. Conf. on Cancer, F. R. Moulton, Editor, A. A. A. S., Washington, D. C., 1945, pp. 267–283.

(10) The authors are indebted to Mr. Ralph Pyke of the Chemistry Department and Dr. Melvin J. Swenson of the School of Veterinary Medicine, Kansas State College, for the histological examination.