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Studies on Carcinolytic Compounds. I. 6,7-Dichloro-9-(1′-D-sorbityl)-isoalloxazine

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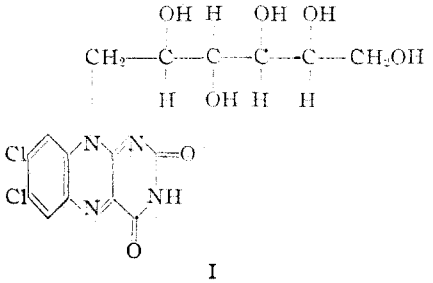
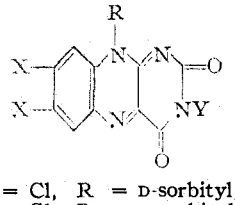
A riboflavin analog, 6,7-dichloro-9-(1′-D-sorbityl)-isoalloxazine (I), has been found to be



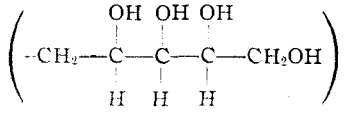
I

effective in producing regression of established lymphosarcoma (6C3H-ED) implants in mice of the C3H strain. Previously, the regression of lymphosarcoma in mice deficient in riboflavin had been reported[1]; in some cases the mice were rendered deficient in riboflavin by feeding one of the riboflavin antagonists, isoriboflavin[2] or galactoflavin[3] along with a diet deficient in riboflavin. The inhibition ratios of isoriboflavin (5,6-dimethyl-9-(1′-D-ribityl)-isoalloxazine) and galactoflavin (6,7-dimethyl-9-(1′-D-dulcityl)-isoalloxazine) to riboflavin are approximately 40 to 1.[4] The present work was begun in an attempt to determine whether new compounds might be synthesized which would be more effective in causing the regression of lymphosarcomas and which might be of interest in other cancer investigations. Compounds having a more favorable riboflavin inhibition ratio might also be found. The first compounds are five isoalloxazine derivatives, I, II, III, IV and V, which are described in this paper.

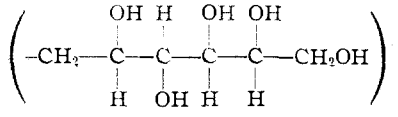


I, X = Cl, R = D-sorbityl, Y = H
II, X = Cl, R = L-arabityl, Y = H
III, X = Cl, R = D-dulcityl, Y = H
IV, X = CH_3, R = D-ribityl, Y = CH_3
V, X = Cl, R = D-dulcityl, Y = CH_3

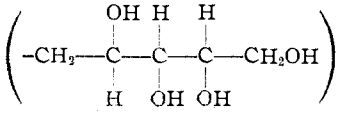
In three of the compounds prepared, the methyl groups of riboflavin were replaced with chloro groups and the D-ribityl



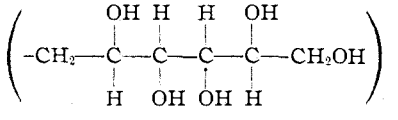
side chain was replaced with D-sorbityl



L-arabityl



and D-dulcityl



The desirability of synthesizing these isoalloxazines was based partly upon the reported antagonism of 6,7-dichloro-9-(1′-D-ribityl)-isoalloxazine[5] to riboflavin.

6,7-Dichloro-9-(1′-D-sorbityl)-isoalloxazine (I), 6,7-dichloro-9-(1′-L-arabityl)-isoalloxazine (II) and 6,7-dichloro-9-(1′-D-dulcityl)-isoalloxazine (III) were synthesized by condensations of alloxan with 2-amino-4,5-dichloro-N-(1′-D-sorbityl)-aniline, 2-amino-4,5-dichloro-N-(1′-L-arabityl)-aniline and 2-amino-4,5-dichloro-N-(1′-D-dulcityl)-aniline, respectively. These aminoanilines were prepared by hydrogenation of the corresponding nitroanilines, which were synthesized by condensation of 1,2-dinitro-4,5-dichlorobenzene with the glycamines, D-glucamine, L-arabinamine and D-galactamine.

The required intermediate sugar derivatives, D-glucamine, L-arabinamine and D-galactamine, were prepared by this procedure: Hydrogenation of D-glucose, L-arabinose and D-galactose in liquid ammonia over a Raney nickel catalyst at 85° at 2,000 p.s.i. for a period of about two hours yielded the corresponding glycamines.[5a] It was found that under the conditions used, a small amount of water is necessary for the reduction to proceed. When the liquid ammonia is collected in a glass liner in Dry Ice, exposed to the air, sufficient water is absorbed by the ammonia; when the ammonia is collected in a closed steel bomb, the glycamine is not produced in a satis-

(1) Stoerk and Emerson, *Proc. Soc. Exp. Biol. and Med.*, **70**, 703 (1949).

(2) Emerson and Tishler, *ibid.*, **55**, 184 (1944).

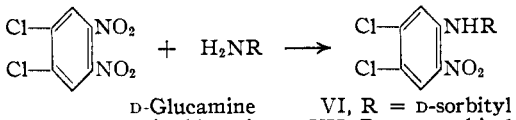
(3) Emerson, Wurtz and Johnson, *J. Biol. Chem.*, **160**, 165 (1945).

(4) Private communication from Dr. Gladys A. Emerson.

(5) Kuhn, Weygand and Möller, *Ber.*, **76B**, 1044 (1943).

(5a) Flint and Salzberg, U. S. Patent 2,016,962 (1932).

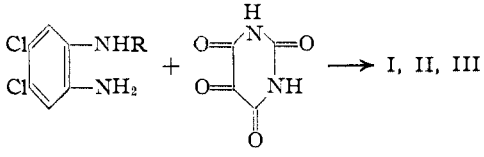
factory yield unless a small amount (3%) of water has been added to the ammonia. The yields of the three glycamines averaged 54% on runs using 150 g. of the aldose.



D-Glucamine VI, R = D-sorbityl
L-Arabinamine VII, R = L-arabityl
D-Galactamine VIII, R = D-dulcityl

The condensation of the glycamines with 1,2-dinitro-4,5-dichlorobenzene proceeded rapidly in aqueous ethanol at about 80° to give high yields of the nitroanilines, 2-nitro-4,5-dichloro-N-(1′-D-sorbityl)-aniline (VI), 2-nitro-4,5-dichloro-N-(1′-L-arabityl)-aniline (VII) and 2-nitro-4,5-dichloro-N-(1′-D-dulcityl)-aniline (VIII). The rapid rates of reaction of these glycamines with 1,2-dinitro-4,5-dichlorobenzene are in contrast to the slow rate at which D-ribamine has been reported[5] to react. The condensation of other glycamines with the dinitrobenzene is being studied.

Reduction of the nitroanilines VI, VII and VIII was accomplished by hydrogenation in acetic acid over a palladium–Darco or platinum catalyst. In glacial acetic acid, the hydrogenation did not proceed to completion, but in acetic acid containing 17% water, the hydrogenation was complete in about one hour. After the catalyst was removed, the solution of the diamine in acetic acid was added to a suspension of alloxan and boric acid in acetic acid[6] to form the isoalloxazines I, II and III.



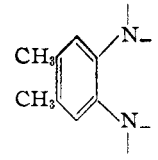
3-Methylriboflavin[7] (IV) was prepared from 2-amino-4,5-dimethyl-N-(1′-D-ribityl)-aniline[8] by a condensation with methylalloxan.[9] 3-Methyl-6,7-dichloro-9-(1′-D-dulcityl)-isoalloxazine (V) was prepared by a condensation of 2-amino-4,5-dichloro-N-(1′-D-dulcityl)-aniline, which was prepared in acetic acid as described above, with methylalloxan.

We are indebted to Dr. Gladys A. Emerson and Dr. Herbert C. Stoerk of the Merck Institute for Therapeutic Research for reports on the activity of the five riboflavin analogs in enhancing regression of the lymphosarcoma implants in mice maintained on a riboflavin deficient diet. 6,7-Dichloro-9-(1′-D-sorbityl)-isoalloxazine (I) was found to be effective in producing regression of established lymphosarcoma (6C3H-ED) implants in mice of the C3H strain; in contrast, 6,7-dichloro-9-(1′-L-arabityl)-isoalloxazine (II) showed only questionable activity, while 6,7-dichloro-9-(1′-D-dulcityl)-isoalloxazine (III), 3-methylriboflavin (IV) and 6,7-dichloro-3-methyl-9-(1′-D-dulcityl)-isoalloxazine (V) were ineffective. These compounds were administered orally at a level equivalent to 1 mg. of riboflavin per day per mouse for seven days; therapy was initiated two to thirteen days post transplant. At the conclusion of this period, the analog was discontinued and 8 μg. of riboflavin was fed daily.

Compounds I and IV were also tested by Dr. Emerson for antiriboflavin activity in rats and showed little or no activity.

Miss Muriel Caswell of the Microbiology Department has kindly assayed the analogs for riboflavin activity and for riboflavin inhibition. *Lactobacillus casei* was used as the test organism. None of the compounds showed inhibition of the growth of *L. casei* when added in 500 times the concentration of riboflavin contained in the medium. Only 3-methylriboflavin had slight riboflavin activity, an estimated 0.09%.

While exhibiting no significant inhibition of riboflavin microbiologically and *in vivo* in rats, compound I nevertheless caused regression of the lymphosarcoma. Even though this compound and riboflavin are isoalloxazine derivatives, the mechanism of regression of the lymphosarcoma need not necessarily occur through riboflavin inhibition. On the basis of chemical structure, this isoalloxazine derivative, I, is related to vitamin B_{12} and to L-lyxoflavin[10] in addition to riboflavin, since all of the latter three substances have the common moiety



Experimental

1,2-Dinitro-4,5-dichlorobenzene.—1,2-Dichlorobenzene (Eastman Organic Chemicals) was nitrated with a mixture of fuming nitric and fuming sulfuric acids by the procedure described by Hartley and Cohen[11] except that the nitration mixture was stirred. After three runs had been completed successfully, a fourth run became strongly exothermic after the reactants were mixed, and a violent evolution of gases occurred. A fifth run also approached a violent rate, but was moderated by use of an efficient ice-bath. The cause of this variation of behavior of the nitration is not known.

Preparation of Glycamines.—D-Glucamine was synthesized by the following procedure, which is representative of the procedures used. To 150 g. of D-glucose in an open glass tube cooled in a Dry Ice-bath, 400 ml. of liquid ammonia and four teaspoonfuls of Raney nickel catalyst were added. The tube and contents were placed in a steel bomb and hydrogenated at about 2,000 p. s. i. at 85° for two hours and fifteen minutes. The ammonia was allowed to evaporate, the residue was dissolved in 500

(6) Kuhn and Weygand, *Ber.*, **68**, 1282 (1935).
(7) Kuhn, Reinemund, Weygand and Ströbele, *ibid.*, **68**, 1765 (1935).
(8) Karrer and Meerwein, *Helv. Chim. Acta*, **19**, 264 (1936).
(9) Biltz, *Ber.*, **46**, 3659 (1912).
(10) Pallares and Garza, *Arch. Biochem.*, **22**, 63 (1949).
(11) Hartley and Cohen, *J. Chem. Soc.*, **85**, 865 (1904).

TABLE I

NITROANILINES

	Compound	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found	M. p.,[a] °C.
VI	$C_{12}H_{16}N_2O_7Cl_2$	38.83	38.80	4.35	4.31	7.55	7.64	193–195
VII	$C_{11}H_{14}N_2O_6Cl_2$	38.73	38.76	4.14	3.97	8.21	8.40	235–237
VIII	$C_{12}H_{16}N_2O_7Cl_2$	38.83	38.43	4.35	3.96	7.55	7.56	236–238

[a] All melting points were determined on a micro-block.

TABLE II

ISOALLOXAZINES

	Compound	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found	M. p.,[a] °C.
I	$C_{16}H_{16}N_4O_7Cl_2$	42.97	42.86	3.61	3.51	12.53	12.73	237–243
II	$C_{15}H_{14}N_4O_6Cl_2$	43.18	42.91	3.38	3.37	13.43	13.41	287–293
III	$C_{16}H_{16}N_4O_7Cl_2 \cdot H_2O$	41.30	41.31	3.90	3.89	12.04	11.99	267–272
	$C_{16}H_{16}N_4O_7Cl_2$	42.97	43.38	3.61	3.77	...	...	.....[b]
IV	$C_{18}H_{22}N_4O_6 \cdot 2H_2O$	50.70	50.40	6.15	5.64	13.14	13.00	268–272
	$C_{18}H_{22}N_4O_6$	55.38	55.57	5.68	5.52	14.35	14.51	.....[c]
V	$C_{17}H_{18}N_4O_7Cl_2$	44.26	44.39	3.93	4.13	12.15	12.02	275–290

[a] All melting points were determined on a micro-block. [b] This analysis was on a sample dried to constant weight at 140°. [c] This analysis was on a sample dried to constant weight at 100°.

ml. of water, the catalyst was removed by filtration, and the filtrate was concentrated *in vacuo* to an oil. Crystallization of the oil from methanol–water yielded 101 g. of D-glucamine,[12] m. p. 122–126°, 83% pure (by titration).

Similarly, from 150 g. of L-arabinose, 81 g. of L-arabinamine,[13] m. p. 96–98°, was obtained, and was 94% pure as determined by titration. From 150 g. of D-galactose, 100 g. of D-galactamine,[14] m. p. 140–142°, 83% pure, was obtained.

The effect of water on the hydrogenation is indicated by the following two experiments.[15] To 540 g. of D-glucose and two tablespoonfuls of Raney nickel catalyst in a closed steel bomb at room temperature was added 1,350 ml. of liquid ammonia, and the mixture was hydrogenated as described above. From this reaction, 285 g. of an impure product, m. p. 110–115° with effervescence at 118°, was isolated; attempts to purify this product were unsuccessful. A similar hydrogenation to which 25 ml. of water had been added yielded 325 g. of D-glucamine, m. p. 120–125°.

Preparation of Nitroanilines (VI, VII and VIII).—A synthesis of 2-nitro-4,5-dichloro-N-(1′-D-dulcityl)-aniline is reported as representative of the method used.

A solution of 15 g. of 1,2-dichloro-4,5-dinitrobenzene in 150 ml. of 80% ethanol at 80° was added to a solution of 26 g. of 83% D-galactamine in 150 ml. of 80% ethanol at 80°. Crystals separated in about one minute, the mixture was cooled in a Dry Ice-bath, the crystals were collected on a filter, and were washed with alcohol and with ether. The product was dried *in vacuo* to give 16.2 g. of 2-nitro-4,5-dichloro-N-(1′-D-dulcityl)-aniline, m. p. 222–232° (micro-block). This product was suitable for use in preparing the isoalloxazine. A sample recrystallized from ethanol–water melted at 236–238° (micro-block). The nitroanilines are described in Table I.

Preparation of Dichloroisoalloxazines I, II and III.—A synthesis of 6,7-dichloro-9-(1′-D-sorbityl)-isoalloxazine (I) is described to illustrate the general method used to prepare compounds I, II and III. A solution of 7.0 g. of 2-nitro-4,5-dichloro-N-(1′-D-sorbityl)-aniline in 120 ml. of acetic acid and 25 ml. of water was hydrogenated over 0.8 g. of platinum oxide catalyst for forty-five minutes at about 35 p. s. i. The solution was filtered into a suspension of 3.7 g. of alloxan monohydrate and 8.4 g. of boric acid in 350 ml. of acetic acid. The mixture was left at room temperature for three days, and was concentrated *in vacuo* to an amorphous residue that was dissolved in 50 ml. of 18% hydrochloric acid. A few drops of "Superoxol" was added to the acid solution; after fifteen minutes 50 ml. of water was added.[16] A precipitate formed which was collected on a filter, washed with water, and dried *in vacuo* to give 5.9 g. of 6,7-dichloro-9-(1′-D-sorbityl)-isoalloxazine, m. p. 225–230° (micro-block). Three recrystallizations from 18% hydrochloric acid–water raised the melting point to 237–243°, with softening at 230° (micro-block). The isoalloxazines are described in Table II.

Summary

6,7-Dichloro-9-(1′-D-sorbityl)-isoalloxazine has been synthesized and has been found to be effective in producing regression of established lymphosarcoma implants in mice. 6,7-Dichloro-9-(1′-L-arabityl)-isoalloxazine, 6,7-dichloro-9-(1′-D-dulcityl)-isoalloxazine and 3-methyl-6,7-dichloro-9-(1′-D-dulcityl)-isoalloxazine were also synthesized, but showed little or no activity. 3-Methylriboflavin also showed no activity.

Hydrogenation of D-glucose, L-arabinose and D-galactose in liquid ammonia over a nickel catalyst has been found to be a convenient procedure for the preparation of the glycamines, D-glucamine, L-arabinamine and D-galactamine, respectively.

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(12) Maquenne and Roux, *Compt. rend.*, **132**, 980 (1901), report m. p. 127°.

(13) Roux, *ibid.*, **136**, 1079 (1903), reports m. p. 98–99°.

(14) Roux, *ibid.*, **135**, 691 (1902), reports m. p. 139°.

(15) These experiments were carried out by Mr. J. F. McPherson.

(16) Pasternak and Brown, U. S. Patent 2,324,800; *C. A.*, **38**, 221 (1944).