portion weighed 0.17 g. and melted after one crystallization from methyl alcohol at 360–372°.

The ether solution was extracted with aqueous sodium hydroxide, which removed a small amount of acidic material that was not investigated. Evaporation of the ether gave 1.74 g. of a neutral fraction which was crystallized from methyl alcohol to give 0.56 g., m.p. 362–372°, identical with the ether-insoluble fraction. Concentration of the mother liquor gave 0.9 g. of crystals melting at 170–340°. After extraction with hot carbon tetrachloride, about one-fifth was insoluble and melted after crystallization from methyl alcohol at 354–364°. Acetylation gave a product, m.p. 280–282°, that did not depress the melting point of diacetylechinocystic acid lactone. Hence the original fraction is identical with the high-melting product formed by the action of alcoholic hydrogen chloride on echinocystic acid.

The four-fifths soluble in carbon tetrachloride, after several crystallizations from methyl alcohol and from acetone, melted at 191–193°. It gave a yellow color with tetranitromethane and was transparent in the ultraviolet. The melting point was not depressed when the compound was mixed with either methyl echinocystate or with methyl anhydroechinocystate, nor did mixing of the last two compounds result in a lowering of their melting points. However, when the fraction was acetylated it depressed the melting point of methyl diacetylechinocystate but not that of methyl acetylanhydroechinocystate. Moreover, hydrogenation of the acetate gave a product, m.p. 213–215°, that did not depress the melting point of methyl acetyloleanolate. Hence the chief action of the alcoholic hydrogen chloride on methyl echinocystate was to dehydrate it to methyl anhydroechinocystate.

The initial ether-insoluble fraction, m.p. 360–372°, is different from the carbon tetrachloride-insoluble fraction, m.p. 354–364°, since acetylation gives a product, m.p. 308–310°, that lowers the melting point of diacetylechinocystic acid lactone. No satisfactory formulas could be deduced from the analyses of either the original compound or its acetate.

Treatment of methyl diacetylechinocystate with alcoholic hydrogen chloride gave methyl anhydroechinocystate and the material, m.p. 350–360°, which on acetylation gave diacetylechinocystic acid lactone. None of the second high melting product was obtained.

Oxidation of 2-Acetylechinocystic Acid.—Oxidation of 2-acetylechinocystic acid under the same conditions used to prepare isonorechinocystenedione gave a product which, after several crystallizations from methyl alcohol, melted at 290–295° in an evacuated capillary tube; $[\alpha]^{24}D$ −90°; λ_{max} 300 mμ, log ε 1.98. The analysis indicates that the product is not the expected 2-acetylnorechinocystenone but is the acetyl diketo lactone of echinocystic acid (X).

Anal. Calcd. for $C_{32}H_{46}O_6$: C, 72.96; H, 8.80. Found: C, 72.53; H, 8.87.

STANFORD, CALIFORNIA

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF MERCK & CO., INC.]

Carcinolytic Compounds. III. 9-(1′-Glycityl)-isoalloxazines

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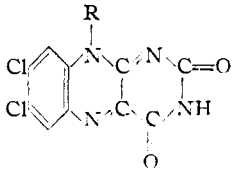
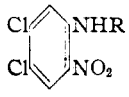
Four riboflavin analogs, 6,7-dichloro-9-(1′-D-mannityl)-isoalloxazine, 6,7-dichloro-9-(1′-D-arabityl)-isoalloxazine, 6,7-dichloro-9-(1′-D-xylityl)-isoalloxazine and 9-(1′-D-sorbityl)-isoalloxazine have been prepared and have been found to be ineffective in enhancing the rate of regression of established lymphosarcoma implants in mice. 6,7-Dichloro-9-(1′-D-ribityl)-isoalloxazine seemed to show slight activity. The rate of condensation of glycamines with 1,2-dinitro-4,5-dichlorobenzene to produce substituted nitroanilines has been shown to be markedly dependent upon the configuration of the glycamines.

Subsequent to the discovery of the activity of 6,7-dichloro-9-(1′-D-sorbityl)-isoalloxazine (I)[1] in enhancing regression of lymphosarcoma implants in mice, four additional dichloroglycitylisoalloxazines and one isoalloxazine containing no chlorine have been prepared.



I, R = D-sorbityl
II, R = D-mannityl
III, R = D-arabityl
IV, R = D-xylityl
V, R = D-ribityl

The dichloroisoalloxazines, 6,7-dichloro-9-(1′-D-mannityl)-isoalloxazine (II), 6,7-dichloro-9-(1′-D-arabityl)-isoalloxazine (III) and 6,7-dichloro-9-1′-D-xylityl)-isoalloxazine (IV), were synthesized by the general procedure described previously.[1] D-Mannamine, D-arabinamine[2] and D-xylamine,[2] were prepared by hydrogenation of the corresponding sugars in liquid ammonia over a nickel catalyst. These glycamines were allowed to react with 1,2-dinitro-4,5-dichlorobenzene in aqueous alcohol to produce 2-nitro-4,5-dichloro-N-(1′-D-mannityl)-aniline (VI), 2-nitro-4,5-dichloro-N-(1′-D-arabityl)-aniline (VII)[2] and 2-nitro-4,5-dichloro-N-(1′-D-xylityl)-aniline (VIII).[2]



VI, R = D-mannityl
VII, R = D-arabityl
VIII, R = D-xylityl

A striking difference was observed in the rate of reaction of D-mannamine with 1,2-dinitro-4,5-dichlorobenzene as compared with the rate at which D-arabinamine and D-xylamine reacted. The latter two gave good yields of the substituted anilines (VII and VIII) in one hour at 90°; under the same conditions with D-mannamine, no reaction was detectable, and after 18 hours at 90° only the dinitrodichlorobenzene was isolated. At 140° for four hours, a reaction occurred and the mannityl-nitroaniline (VI) was produced. This rate of reaction of D-mannamine with 1,2-dinitro-4,5-dichlorobenzene is similar to the slow rate at which D-ribamine[2,3] also reacts. The glycamines D-glucamine, L-arabinamine and D-galactamine reacted with 1,2-dinitro-4,5-dichlorobenzene in one minute at 80° to yield the substituted nitroanilines.[1]

(1) F. W. Holly, E. W. Peel, R. Mozingo and K. Folkers, THIS JOURNAL, **72**, 5416 (1950).

(2) F. W. Holly, E. W. Peel, J. J. Cahill and K. Folkers, *ibid.*, **73**, 332 (1951).

(3) R. Kuhn, F. Weygand and E. F. Möller, *Ber.*, **76B**, 1044 (1943).

TABLE I

ISOALLOXAZINES

		Calcd., %			Found, %			
		C	H	N	C	H	N	M.p.,[a] °C.
II	$C_{16}H_{16}N_4O_7Cl_2$	42.97	3.61	12.53	42.46	3.82	12.66	258–261[b]
III	$C_{15}H_{14}N_4O_6Cl_2$	43.18	3.38	13.43	42.91	3.49	13.22	267–269
IV	$C_{15}H_{14}N_4O_6Cl_2$	43.18	3.38		43.10	3.52		268–272[c]
IX	$C_{16}H_{18}N_4O_7$	50.79	4.79	14.73	50.73	4.84	14.91	299–300[d]

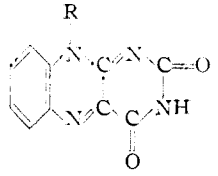
[a] All melting points were determined on a micro-block. [b] Recrystallized from pyridine–ether. [c] Calcd.: Cl, 17.00. Found: Cl, 16.92. [d] Nine crystallizations from acetic acid–water.

Since D-arabinamine, L-arabinamine,[1] D-xylamine, D-glucamine[1] and D-galactamine[1] react rapidly with 1,2-dinitro-4,5-dichlorobenzene, while D-ribamine and D-mannamine react slowly, it appears empirically from this small series that glycamines having the C_2- and C_3-hydroxyl groups *trans* react rapidly and that glycamines having the C_2- and C_3-hydroxyl groups *cis* react slowly with 1,2-dinitro-4,5-dichlorobenzene to yield a substituted nitroaniline.

Hydrogenation of the nitroanilines VI, VII and VIII, over a palladium catalyst in acetic acid containing 17% of water gave colorless solutions which, after removal of the catalyst, were allowed to react with alloxan, yielding isoalloxazines II, III and IV.

6,7-Dichloro-9-(1′-D-ribityl)-isoalloxazine (V) was synthesized as described[3] by condensation of D-ribamine with 1,2-dinitro-4,5-dichlorobenzene to form 2-nitro-4,5-dichloro-N-(1′-D-ribityl)-aniline, which was converted into the isoalloxazine (V).

9-(1′-D-Sorbityl)-isoalloxazine (IX) was synthesized by hydrogenation of 2-nitro-N-(1′-D-sorbityl)-aniline and condensation of the reduced product with alloxan. 2-Nitro-N-(1′-D-sorbityl)-aniline was prepared by a reaction of 2-nitrochlorobenzene with D-glucamine in pyridine.[4]



IX, R = D-sorbityl

For studies of the effect of the isoalloxazines on lymphosarcoma in mice, we are indebted to Dr. Gladys Emerson of the Merck Institute for Therapeutic Research. The tests were carried out as described previously.[1] 6,7-Dichloro-9-(1′-D-ribityl)-isoalloxazine showed slight activity and the remaining dichloroisoalloxazines were inactive or showed only questionable activity in enhancing regression of established lymphosarcoma (C3H-ED) implants in mice of the C3H strain subjected to transient riboflavin deprivation.

(4) R. Kuhn and F. Weygand, *Ber.*, **67**, 1939 (1934).

9-(1′-D-Sorbityl)-isoalloxazine (IX) was ineffective in the above test with lymphosarcoma.

Experimental[5]

D-Ribamine.—Hydrogenation of D-ribose in liquid ammonia by the procedure described[1] previously for synthesis of glycamines yielded D-ribamine, which was used as an oil without purification.

2-Nitro-4,5-dichloro-N-(1′-D-mannityl)-aniline (VI).—A solution of 14.2 g. (0.054 mole) of 70% D-mannamine prepared as described for other glycamines[1,2] in a minimum quantity of hot 80% ethanol was added to a solution of 11.9 g. (0.05 mole) of 1,2-dichloro-4,5-dinitrobenzene[1] in a minimum quantity of hot 80% ethanol, and the mixture (total volume = 250 ml.) was heated on a steam-bath for five minutes. The solution was cooled; 1,2-dinitro-4,5-dichlorobenzene crystallized from the solution, and was added back to the solution and the solution was refluxed for 18 hours. When the solution was cooled, 1,2-dinitro-4,5-dichlorobenzene separated. The mixture therefore was heated at 140° under nitrogen in a steel bomb for four hours. The solution was cooled; the orange precipitate which formed was collected on a filter, washed with ether, and dried to give 8 g. of 2-nitro-4,5-dichloro-N-(1′-D-mannityl)-aniline (VI), m.p. 173–177° (micro-block). A sample recrystallized four times from ethanol melted at 177–178° (transition at 173–176°, micro-block).

Anal. Calcd. for $C_{12}H_{16}N_2O_7Cl_2$: C, 38.83; H, 4.35; N, 7.55. Found: C, 39.23; H, 4.56; N, 7.81.

2-Nitro-4,5-dichloro-N-(1′-D-arabityl)-aniline (VII) and 2-nitro-4,5-dichloro-N-(1′-D-xylityl)-aniline (VIII) were prepared by the previously described procedure.[2]

2-Nitro-N-(1′-D-sorbityl)-aniline.—To a solution of 50 g. of 2-nitrochlorobenzene in 150 ml. of pyridine, was added 50 g. of 83% D-glucamine; the solution was refluxed for four hours.[4] The mixture was steam distilled, the residue was extracted with chloroform and concentrated to a smaller volume. After the aqueous solution had remained at 0° for 15 hours, crystals had separated. They were collected on a filter and washed with methanol. Six grams of 2-nitro-N-(1′-D-sorbityl)-aniline was obtained, m.p. 153–159° (micro-block). This material was satisfactory for subsequent reactions. A sample crystallized three times from methanol melted at 158–161° (micro-block).

Anal. Calcd. for $C_{12}H_{18}N_2O_7$: C, 47.67; H, 6.00; N, 9.27. Found: C, 47.94; H, 6.10; N, 9.20.

Preparation of Isoalloxazines (II, III, IV and IX).—The isoalloxazines were prepared by the general method used for the preparation of other isoalloxazines[1] and are described in Table I.

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(5) We are indebted to Mr. Richard Boos and his associates for the microanalyses.