Table II

Mixture	Labile pigment		Xanthommatin		Dihydroxan.		I		II	
ButOH-AcH-0.5N HCl (60:15:25)	450	430	460	390	498	385	450	430	450	430
2N HCl	500	460	470	390	500 (I)	470	495	462	495	462
							435 (I)		430 (I)	

(I) = inflection

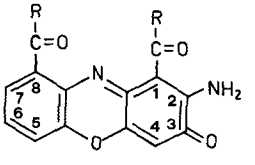
At room temperature, this labile pigment with UV-maxima at 450 and 430 nm is rapidly converted into a red substance with UV-maxima at 498 and 385 nm which, in turn, is transformed into a product with UV-maxima at 460 and 385 nm. It should be noted that the presence of an isosbestic point indicates the complete transformation of the labile pigment into the product with maxima at 498 and 385 nm. This isosbestic point is also observed in the extracts from all animals reported in Table I. In all the cases examined, both products of transformation were isolated and purified by chromatography column on carboxy-methyl-cellulose (Whatman CM 23) using buthanol-acetic acid-H_2O (60:15:25 v/v) as eluent. They were identified, as dihydroxanthommatin and xanthommatin, by comparison of their chromatographic properties with those of authentic samples[4].

The conversion of labile pigment into dihydroxanthommatin did not involve an oxidative reaction as it occurred also in the presence of reducing agents (SO_2, ascorbic acid) which of course inhibited the subsequent transformation of dihydroxanthommatin into xanthommatin.

As shown in Table I, after exhaustive extraction of labile pigment from the animal tissues, generally no xanthommatin can be detected.

Taking into consideration these results, we suggest that xanthommatin probably arises from the labile precursor. In order to obtain information on the nature of this labile ommochrome, we compared its electronic spectrum with those of two phenoxazinic substances obtained by oxidation of 3-hydroxyanthranilic acid and its methylester.

A solution of 0.04 mole of $K_3[Fe(CN)_6]$ in 20 ml of phosphate buffer pH 6.8 was added to a solution of 1.5 g (0.01 mole) of 3-hydroxyanthranilic acid in 10 ml of the same buffer, at 25 °C with agitation. Stirring was continued for 1 h, after which the solution, acidified with acetic acid, was extracted with ethyl acetate. The organic phase was concentrated and purified by chromatography column 2 × 30 cm on polyamide (Macerey, Nagel and Co.) using benzene as eluent. The fraction containing product I, which emerged from the column after about 200 ml, was identified as cinnabarinic acid[3] by mass spectroscopy ($M^+ = 320$).



I R=OH
II R=OCH_3
III R=CH_2–CH(NH_2)–COOH

The ester II[5] of cinnabarinic acid ($M^+ = 328$) was characterized by spectral data. NMR-spectrum in CD_3SOCD_3 shows signals due to OCH_3 groups at δ 3.85 and δ 3.90 (each 3H, s) to C(4)-proton at δ 6.5 (1H, s), to aromatic protons at δ 7.6 (3H, s) and to aminic protons at δ 7.8 (2H, by D_2O exchanged). IR-spectrum shows the C(8)-carbomethoxy C = O stretching band at 1724 cm^{-1}; C(1)-carbomethoxy C = O at 1670 cm^{-1}; C = O quinonic at 1640 cm^{-1}.

The Table II shows the UV-maxima of the labile pigment in two extracting mixtures and those of products I and II, dihydroxanthommatin and xanthommatin in the same solvents.

The values reported in Table II indicate that the behaviour of the chromophore of the labile pigment corresponds to those of compounds I and II. These results support the hypothesis that the pigment has a chromophore of type III. In nature the two uncyclized aminoacidic chains are plausibly stabilized.

Riassunto. Un nuovo ommocromo labile è stato rinvenuto in alcuni invertebrati. Sulla base del suo comportamento chimico e spettrale si puó ritenere che sia un precursore della xantommatina.

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Imidazole Nucleoside Analogues Possessing a Non-Glycosidic Link between Sugar and Base

A number of analogues of most of the important naturally occurring imidazole ribofuranosides have been prepared[1,2,3] for use in studies of the *de novo* biosynthesis of purines and as potential anti-virus and anti-cancer compounds. Most of them have the same carbohydrate component as the natural purine precursors and all contain a glycosidic link between sugar and base. Recently[4] we synthesized an imidazole nucleoside (I) in which

Table I. Stereochemical comparison of nucleoside analogue (III) with the naturally occurring nucleoside (II) corresponding to AICAR. Interatomic distances between various atoms were measured on Dreiding stereomodels

Analogue (III) (in boat confirmation)		Nucleoside (II)	
Atoms	Distance (Å) apart	Atoms	Distance (Å) apart
C_5–N_1	3.60	C_6–N_2	3.48
C_4–C_1	2.44	C_5–C_2	2.74
N_1–O_3	4.48	N_2–O_4	4.25
N_1–O_2	3.56	N_2–O_3	3.56
O_5–C_1	1.43	O_6–C_2	2.36
O_5–N_2	2.44	O_6–N_2	2.80
O_5–C_2	2.52	O_6–C_3	2.88
O_5–C_3	2.52	O_6–C_4	2.40
C_4–C_2	2.52	C_5–C_3	2.52
C_4–O_2	3.44	C_5–O_3	3.80
C_4–N_1	3.16	C_5–N_2	3.60

Table II. T.l.c. data for various nucleoside analogues

	RF value on cellulose thin layers Solvent system [a]						
	A	B	C	D	E	F	G
II	0.22	0.41	0.43	0.38	–	–	–
III	0.13	0.25	–	0.38	0.43	0.40	0.30
V	0.25	0.40	0.51	0.53	0.70	0.30	–
IX	0.12	0.23	0.43	0.37	0.55	0.10	–
VIII	0.20	0.50	0.48	0.35	0.21	–	–
VII	0.12	0.23	0.45	0.40	0.54	0.15	0.29
X	0.23	–	0.53	0.50	0.66	0.25	–
XI	0.16	0.30	0.49	0.42	–	0.22	–

[a] A, *n*-butanol (18)-water (83); B, ammonium citrate (pH 4.4) (18)-ethanol (82); C, *n*-butanol (4)-ethanol (1)-water (5) (upper layer); D, *iso*-propanol (4)-0.2 *N* ammonium hydroxide (1); E, saturated $NH_4H\,CO_3$ solution; F, *iso*-propanol (4)-water (1); G, *iso*-propanol (6)-water (2)-ammonia (0.880) (3).

the heterocyclic ring is connected to the 2′-position of the sugar. Compounds of this class should be stable in the presence of glycoside-splitting enzymes and might be expected to be useful metabolic inhibitors; however, the examples we reported are stereochemically dissimilar to the natural ribofuranosides. Our work has now been extended to provide nucleoside analogues that are more closely related to the key purine precursor AICAR and the corresponding nucleoside (II) or which are capable of attaining a similar conformation during interactions with enzymes. One example is the altropyranoside derivative (III) which has similar dimensions to AICAR particularly when the less stable boat conformation is considered (Table I).

Altropyranoside (III) was synthesized by treatment of 2-amino-2-*deoxy*-D-altrose with ethyl *N*-[carbomoyl(cyano)methyl] formimidate[5] (IV) at pH 8. The product was extremely sensitive to ice-cold dilute acid, alkali, and even to warm water but it was successfully purified by chromatography on DEAE-cellulose at 4 °C using water as eluant and the eluted material could be freeze-dried without decomposition. The product appeared to be homogeneous on thin layer chromatograms (Table II), showed spectroscopic values consistent with structure (III) (Table III), and gave a purple colour in the BRATTON-MARSHALL test[6] indicative of aminoimidazoles. The compound quickly darkened in dilute acid to produce a black solid (mp > 350 °C) which did not move from the origin on t.l.c. plate and is presumably a polymer.

For in vitro studies nucleotides are often preferred to nucleosides but the acid sensitivity of compound (III) precluded phosphorylation directly. Therefore, the related methyl 2-altropyranoside derivative (V) was synthesized from methyl 2-amino-2-*deoxy*-D-altropyranoside[7] and imidate (IV). This glycoside was much more stable to acid and alkali and could be purified on an ion-exchange column (Table IV) and phosphorylated by the method of

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Table III. Spectroscopic and periodate titration data for nucleoside analogues

Compound	Spectrum in acid λ_{max} (nm)	ε	Spectrum in neutral solution λ_{max} (nm)	ε	Spectrum in alkali λ_{max} (nm)	ε	Spectrum in the Bratton-Marshall test λ_{max} (nm)	ε	Periodate titration mole equivalent of 10_4-consumed
II	246,267	10,200	267	10,300	265–6	10,900	540	26,000	1.01
III	246,276	8,450	266–7	10,200	265–6	10,900	545	15,100	2.98
V	242,267	9,400	265	10,500	265	10,650	552	14,800	1.02
VI	244,268	8,000	267	10,000	268	10,600	550	24,600	1.03
IX	246,267	9,600	267	10,700	267	10,650	550	13,200	2.99
VIII	246,266	9,800	265	10,000	267	10,100	545	12,900	2.05
VII	244,267	9,100	267	11,000	265	11,500	549	14,150	2.33
X	242,267	10,200	265	10,900	267	11,650	545	13.500	0
XI	245,267	11,600	267	10,400	267	11,650	550	21,050	0

Table IV. Purification of nucleoside analogues by column chromatography on ion-exchange resins

Compound	Resin type	Column dimensions	Elution with water at flow rate (ml/h)	Major fraction emerged between (ml)
III	DE 52	32 × 2.0	12	63– 85
IX (picrate) [a]	DE 52	15 × 1.0	15	70– 91
V	AG 1 × 8 (Formate) 200–400 mesh	58 × 3.5	33	116–164
VI	,,	19 × 1.5	48	246–317 [b]
VIII	,,	45 × 3.5	45	324–404
X	,,	16.5 × 3.0	33	146–252
XI	,,	32 × 3.5	33	175–294

[a] a crystalline picrate was formed by adding aqueous picric acid, this was collected, washed, dissolved in water and applied to the column; [b] washed initially with water (112 ml) then eluted with M-formic acid.

TENER[8]. Despite the fact that the 2′-and 4′-hydroxyl groups were not protected during the phosphorylation reaction the 6′-monophosphate (VI) was the only major product, after purification by ion-exchange chromatography it had a similar electrophoretic mobility to AMP and GMP, contained 1 mole equivalent of phosphate, consumed 1 mole equivalent of periodate[9], gave a purple colour in the BRATTON-MARSHALL test and showed the characteristic ultraviolet spectrum of an aminoimidazole.

A number of other 2′-substituted analogues were synthesized from the appropriate amino sugar. Both the mannose derivative (VII) and arabinose derivative (VIII) have a similar stereochemistry to (II). The galactose compound (IX) which is related to the α-anomer[10] of (II) was also prepared. All these compounds mutarotate in water and are unstable in acid although they decompose at a slower rate than compound (III).

The method was also applied to the synthesis of 3′-(amino-imidazole carboxamide) derivatives from the methyl glycosides of 3-amino-3-*deoxy*-D-glucose and 3-amino-3-*deoxy*-D-altrose[11]; as expected, the products (X and XI) did not consume periodate but were otherwise similar to the isomeric 2′-examples (Tables II and III).

Experimental procedures. The 2-amino-2-*deoxy*-D-monosaccharide hydrochloride (270 mg) (or methyl glycoside, etc.) dissolved in a minimum volume of water was adjusted to pH 8.0 with solid potassium bicarbonate and then diluted to 8 ml with methanol. Ethyl *N*-[carbamoyl-(cyano)methyl] formimidate (350 mg) was added and the mixture kept at room temperature for 24 to 48 h. The mixture was evaporated to dryness in a rotary evaporator at 38 °C. The residue was dissolved in methanol and again evaporated then redissolved in water and purified by column chromatography (Table IV). In one case (compound VII) the product crystallized out when the reaction mixture was concentrated and chromatographic purification was unnecessary. Most of the methods used in characterising the products we have described elsewhere[4,10]. Electrophoresis was carried out on a Shandon flat bed apparatus at 10 °C using Whatman 3 mm paper at 2 volts per cm at *pH 1.85* [acetic acid (15)-formic acid (10)-water (255) (v/v)], *pH 9.4* [in NaH CO_3 (56.8) (N · Na_2CO_3 (14.4)-water (929) (v/v)], and *pH 9.1* (1% sodium tetraborate in water); products were detected using a) the BRATTON-MARSHALL spray reagents[12] and b) ammonium molybdate spray reagents for phosphate[13].

Zusammenfassung. Es wird eine Reihe von Imidazolglykosiden und deren Synthese beschrieben. Diese eignen sich als Analoga der natürlichen Nukleoside für biologische Versuche.

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Über das Vorkommen von Carboanhydratase in der Eischale des Huhnes

Die kalkige Eischale des Huhnes besteht zu 97% aus Calziumcarbonat und einer organischen Matrix. An der Eischalenbildung ist Carboanhydratase (E. C. 4.2.1.1.) beteiligt, da dieses Enzym durch die Katalyse der Reaktion $H_2O + CO_2 \rightleftharpoons H_2CO_3$ die Bildungsrate des Anions des Calciumcarbonates beschleunigt[1]. Dieses Enzym ist mit Sicherheit im Uterus von Hühnern nachgewiesen worden,[2–4] wo es nicht strukturgebunden in den Mucosazellen vorliegt. In der Mammillenschicht der Eischalen von Hühnern wiesen ROBINSON und KING[6] Carboanhydratase mittels histochemischer Methoden nach. DIAMANTSTEIN[5] und SCHLÜNS[3] konnten diese Beobachtungen jedoch nicht bestätigen. Wir haben diese Fragestellung erneut aufgegriffen und im Zusammenhang mit der Fraktionierung von Eischalenproteinen bearbeitet[7].

Methodik. Für die Extraktion von Carboanhydratase aus Eischalen wurden Eier (1–2 Tage alt) von HNL-Hybriden verwendet und nach dem von KRAMPITZ und ENGELS[7] beschriebenen Verfahren aufgearbeitet. Besondere Beachtung verdient die Beobachtung, dass allen Pufferlösungen, die zur Extraktion und zur Chromatographie verwendet wurden, 2-Mercaptoäthanol zugesetzt werden musste, um die Aktivität der Carboanhydratase zu erhalten.