can be inferred from our findings, in addition to the large amount of evidence accumulated in the literature. Some of the phytoalexins mentioned by CRUICKSHANK[16] are derived from phenolic acids. KLARMAN[17] isolated phenolic substances from soybeans infected with phytophtora megasperma; so did BIEHN, WILLIAMS and KUĆ[18] after infecting the beans with various fungi.

This paper does not deal with the physiological and toxicological sides of the phenomenon. However, in view of the ultimate aim of human consumption, these aspects have to be kept in mind, as clearly pointed out by SINGLETON and KRATZER[19].

Zusammenfassung. Beim spontanen Bräunen und Erhitzen von gelagerten Soyabohnen bilden sich Phenolsäuren, welche eine aktive Rolle in Entstehen des Lagerschadens spielen und auch als dessen Frühindikator dienen können.

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[16] J. A. M. CRUICKSHANK, A. Rev. Phytopath. *1*, 351 (1963).
[17] W. L. KLARMAN and J. B. SANDFORD, Life Sci. *1*, 1095 (1968).
[18] W. L. BIEHN, J. Kuć and E. B. WILLIAMS, Phytopathology *58*, 1255 (1968).
[19] V. L. SINGLETON and F. H. KRATZER, J. agric. Food Chem. *17*, 497 (1969).
[20] The authors wish to express their appreciation to Prof. A. BONDI for helpful suggestions. They also wish to thank Misses FRIEDA RABINOWITZ and MIRI KEKUN for skilful technical assistance.

Synthesis and Stereospecific Synthesis of some Alkyl-5-Amino-1-Glycofuranosyl Imidazole-4-Carboxylates Related to Intermediates in Purine Nucleotide de novo Biosynthesis

The aminoimidazolecarboxylic acid ribotide C-AIR (I) is an important intermediate in the biosynthesis de novo of purine nucleotides[1], and is of value for the synthesis of acyclic intermediates in the same pathway. We have synthesized the nucleotide previously[2] by phosphorylation of the isopropylidene ribonucleoside (IIa), subsequent removal of the isopropylidene group with acid, and hydrolysis of the ester with alkali. The imidazole ester (IIa) was obtained by the reaction of 2, 3, 5-*tri*-*O*-benzoylribofuranosylamine (III) with the formimidate (IVa), hydrolysis of the derived tribenzoyl nucleoside, and condensation of the resulting nucleoside with dimethoxypropane and tosic acid, or by condensation of the 2, 3,-*O*-isopropylidene ribofuranosylamine tosate (V)[3] with the same reagent (IVa) in the presence of base.

Subsequent reactions of the protonated isopropylidene ribofuranosylamine (V) with (IVb) (prepared by refluxing ethyl-α-amino-α-cyanoacetate with triethyl *ortho*formate in acetonitrile for 45 min) and base, however, gave a mixture of the α- and β-isopropylidene aminoimidazole nucleosides ((VI) and (IIb), respectively), which were very readily separated without chromatography and obtained as crystalline solids (Table I). Closer examination of the earlier reaction of (V) with the methyl ester formimidate (IVa) and base also revealed the presence of the corresponding α-nucleoside.

Table I.

Imidazole nucleoside	Yield (%)	m.p. °C	$[\alpha]_D^{20}$ (C, %)[a]	λ_{max}(nm)[b] [ε]	BRATTON MARSHALL[9] λ_{max}(nm)
(XIV)	75	177–9	−24° (0.4)	266 [12,640]	513
(VI)	22	188–190	−70° (0.3)	267 [11,060]	508
(IIb)	16	180–2	−97° (0.3)	267 [12,900]	512
(IIa)	—	161–2	−100° (0.3)	267 [13,200]	508
(X) [A]	20	225–6	+95° (0.2)	267 [13,000]	518
(XI) [B]	20	190–2	+43° (0.2)	266 [12,700]	508

[a] Measured in DMSO. [b] Slight inflection at 230–240 nm; solvent methanol.

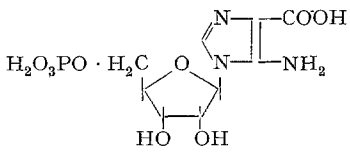
The same mixture of α- and β-nucleosides (VI) and (IIb) was also obtained by a modification of this last synthesis which involved prior reaction of the ribosylamine tosate (V) with ethyl formimidate hydrochloride and triethylamine, and subsequent reaction of the mixture of intermediates (VII), which are presumably formed, with ethyl-α-amino-α-cyanoacetate.

The structure assigned to the methyl β-nucleoside (IIa) was confirmed by elemental analysis, and comparison (TCL, IR, UV, m.p. and mixed m.p.) with material already prepared via the benzoyl intermediate and earlier shown to have the β-configuration by conversion into inosine[2] and into various intermediates in the de novo pathway leading to purine nucleotides.

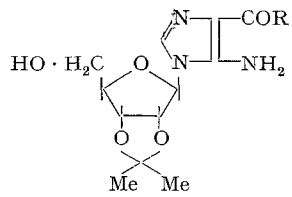
Assignment of the β-structure to the corresponding ethyl ester nucleoside (IIb) was confirmed by elemental analysis, mass spectrum ($M^+ = 327$) and by comparison of optical rotation (Table I), and circular dichroism (Figure 1) measurements with these of the methyl β-nucleoside (IIa). The β-ethyl ester nucleoside (IIb) with ammonia gave the aminoimidazole carboxyamide (IIc) which was identical (TLC in several solvents) with that produced from the β-methyl ester (IIa). The structure assigned to the ethyl α-ester (VI) was confirmed by elemental analysis, mass spectrum ($M^+ = 327$) and the differences in optical rotation (Table I) and circular dichroism (Figure 2) measurements compared to those of the β-ester (IIb). The α-riboside (VI) also differs from the β-anomer in behaviour on thin layer chromatogram, and rate of loss of isopropylidene group in 10% aqueous acetic acid at 100°C (the β-form required 2 h for complete deacetonation compared with $3^1/_2$ h for the α-form; some aglycone was formed in each case).

In addition the assignments agree well with HUDSON's[4] isorotation rules. These rules have been shown to apply to analogous purine nucleosides[5]. The NMR-spectra were not

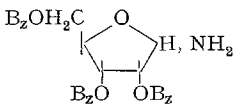
[1] J. G. BUCHANAN and S. C. HARTMAN, Adv. Enzymol. *21*, 119 (1959). – S. C. HARTMAN and J. G. BUCHANAN, A. Rev. Biochem. *28*, 365 (1959). – J. BADDILEY and J. G. BUCHANAN, Q. Rev. chem. Soc. *11*, 329 (1957).
[2] G. SHAW and D. V. WILSON, J. chem. Soc. *1962*, 2937.
[3] N. J. CUSACK and G. SHAW, Chem. Commun. *1970*, 1114.
[4] C. S. HUDSON, J. Am. chem. Soc. *31*, 66 (1909).



(I)

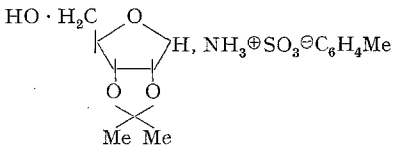


(IIa) R = OMe
(IIb) R = OEt
(IIc) R = NH_2

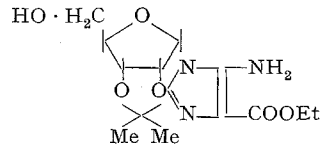


(III)

EtO · CH · N · CH(CN)COOR
(IVa) R=Me
(IVb) R=Et



(V)



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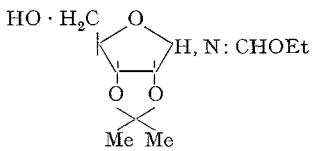

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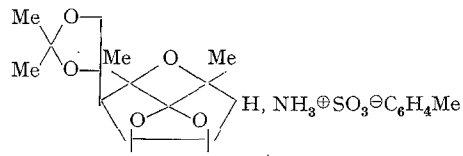

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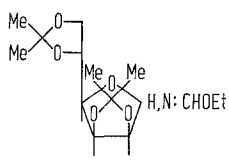

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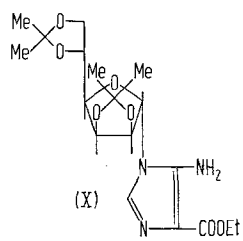
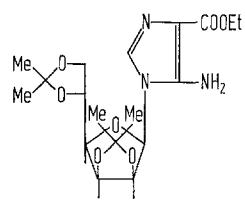


(X)



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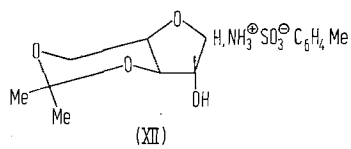
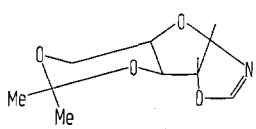
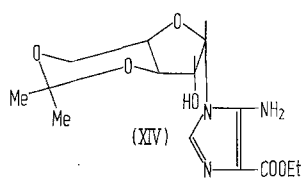


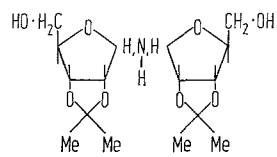
(XII)



(XIII)



(XIV)



(XV)

conclusive in the case of the imidazole ribonucleosides since the $H_{1'}$ protons are split by about the same amount in each case (Table II)[6].

In the same manner a mixture of 2 isopropylidene mannofuranosylaminoimidazole derivatives, A and B (Table I), were obtained by condensation of 2, 3 : 5, 6-*O*-isopropylidene-D-mannofuranosylamine tosate (VIII)[7] with either the formimidate (IVa) and base, or by prior reaction of the mannosylamine tosate with ethyl formimidate hydrochloride and triethylamine and subsequent reaction of the mannosyl formimidates (IX), which are presumably formed, with ethyl α-amino-α-cyanoacetate. The furanose configuration of both isomers was confirmed by removal of the isopropylidene groups with aqueous acetic acid and isolation of formaldehyde as the dimedone derivative in good yield after periodate oxidation of the derived nucleosides[8].

Elemental analyses and mass spectra ($M_A^+ = 397$, $M_B^+ = 397$) agreed well with the structure of ethyl 5-amino-1 (2, 3 : 5, 6-*di*-*O*-isopropylidene-D-mannofuranosyl)imidazole-4-carboxylate. Application of HUDSON's rules to the mannosyl imidazole nucleosides indicate that A can be assigned the α- configuration (X) and B the β-configuration (XI). Further evidence for these assignments is provided by comparison of the shift in the visible spectral maxima of the dyestuffs produced in the BRATTON-

[5] T. R. EMERSON and T. L. V. ULBRICHT, Chemy. Ind. *52*, 2129 (1964). T. L. V. ULBRICHT, J. P. JENNINGS, P. M. SCOPES and W. KLYNE, Tetrahedron Lett. *13*, 695 (1964).
[6] T. NISHIMURA and B. SHIMIZU, Chem. Pharm. Bull. *13*, 803 (1965).
[7] N. J. CUSACK, P. W. RUGG and G. SHAW, Chem. Commun. 190 (1970).
[8] R. E. REEVES, J. Am. chem. Soc. *63*, 1476 (1941).

MARSHALL[9] assay of the mannose nucleosides when compared with those of the ribose analogues (Table I), when a characteristic difference appears according to whether the isopropylidene and imidazole ring systems are in the *cis* or *trans* configuration. The NMR-spectrum of the α-imidazole mannosyl nucleoside (X) shows the H_1' proton as a singlet (Table II), whereas the β-anomer has a very broad singlet showing a tendency to split. This confirms the *trans* H_1', H_2' proton arrangement in the α-form and the *cis* H_1', H_2' proton arrangement in the β-form[10].

In contrast to the above reaction sequences, 3,5-*O*-isopropylidene-D-xylofuranosylamine tosate (XII) with ethyl formimidate hydrochloride and triethylamine gave a readily isolated crystalline compound, m.p. 119–121°C. $[\alpha]_D^{20} = +9.0°$ (C = 2.8% in DMSO) in excellent yield (>65%). We assign the oxazoline structure (XIII) to this compound. Evidence for this structure comes from elemental analysis, and IR-spectra (absence of O–H, but a strong band at 1620 cm^{-1} (C = N), and a doublet at 1380 cm^{-1}; characteristic of the isopropylidene group). The mass spectrum shows a very small mass ion at 199 $(M)^+$ and 200 $(M+1)^+$ but a strong peak at 184 $(M-CH_3)^+$; this pattern is common in these types of isopropylidene sugar derivatives. NMR-spectra in $CDCl_3$ shows a one proton singlet at $\tau = 2.97$ corresponding to the proton of the oxazoline ring, and a one proton doublet at $\tau = 3.8$ corresponding to the glycosidic H_1' proton ($J_{H_1', H_2'} = 6.0 \pm 0.5$ Hz).

In addition the oxazoline reacted smoothly with ethyl-α-amino-α-cyanoacetate to give the xylofuranosyl imidazole (XIV) in excellent yield (Table I) with no evidence for the presence of any β-isomer in the product.

The structure of (XIV) was confirmed by elemental analysis, and mass spectrum ($M^+ = 327$). The α-configuration is assumed because of the mode of synthesis and the absence of any other aminoimidazole nucleoside in the reaction mixture (TLC examination), which implies that no mutarotation occurs at the acyclic intermediate stage (R–N=CH–NH–CH (CN)COOEt where R is the isopropylidene xylofuranosyl moiety).

Earlier work on the condensation of the isopropylidene-ribo-, manno-, and xylofuranosylamine tosates (V), (VIII) and (XII), respectively[3,7], with acyclic β-ethoxy acryloyl urethanes suggested that only a single isomer was being formed, generally in excellent yields of up to 80%. However, it is clear from our current results on aminoimidazoles that mixtures of anomeric isomers are produced. This implies that either the glycofuranosyl tosates are anomeric mixtures or that mutarotation occurs in subsequent reactions, either with the unprotonated glycofuranosylamines or with the acyclic intermediates which are formed in these types of reactions. The formation of a single pure isomer from the oxazoline (XIII) and ethyl-α-amino-α-cyano-acetate suggests that there is little or no mutarotation of the acyclic structures formed in this reaction. In addition the variation in yields of the various α- and β-isomers produced using acryloylurethanes and formimidates would suggest that if, as seems inevitable, mutarotation is occurring, it is happening with the unprotonated glycofuranosylamines which are liberated during the reactions. Confirmation that this is so has come from a study of the rapid change of optical rotation of the unprotonated isopropylidene ribofuranosylamine and from the accompanying ready formation of the corresponding *bis*-ribofuranosylamine (XV)[11] [m.p. 153–154°; $M^+ = 361$; $[\alpha]_D^{20} -144°$ (C = 0.4% DMSO)]. Also preliminary kinetic experiments involving the reaction of the formimidate (IV b) with simple aliphatic amines reveals that the rate is very slow (4–5 h for completion at room temperature) compared with that of the acryloylurethanes which react within seconds of mixing.

The stereospecific synthesis of nucleosides from oxazoline precursors offers a valuable new route to a variety of N-substituted glycosides and these and the various related reactions outlined above are under further investigation.

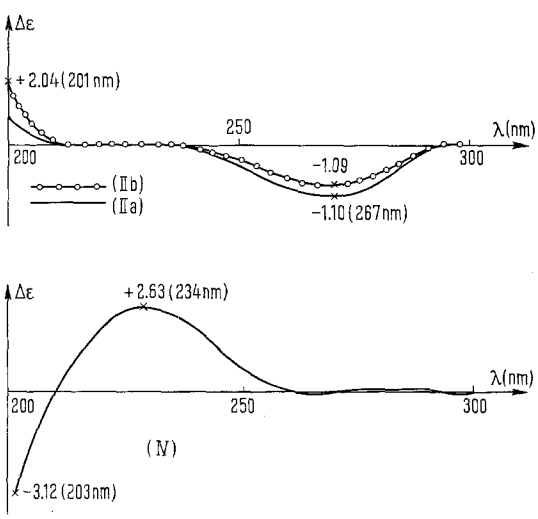


Fig. 1 and 2. Circular dichroism spectra of some anomeric imidazole ribonucleosides. Crosses mark maxima and minima of curves.

Table II. Nuclear magnetic resonance[a] spectra of some aminoimidazole nucleosides

Imidazole nucleoside	τH_1	JH_1', H_2'
(VI)	4.04	3.5±0.5 Hz
(IIb)	4.17	3.5±0.5 Hz
(X) [A]	4.03	Singlet
(XI) [B]	4.39	Broad singlet

[a] Reference tetramethylsilane. Solvent d_6 DMSO:D_2O (4:1).

Zusammenfassung. 5-Amino-Glycofuranosylimidazol-Derivative wurden durch Kondensation der Isopropyliden-glycofuranosylamine mit Äthyl-N-(Cyano-N-Äthoxycarbonylmethyl)formimidat oder durch Reaktion von Äthylformimidat-Hydrochlorid mit den Furanosylaminen, wozu Äthyl-α-amino-α-cyanoacetat hinzugefügt wurde, dargestellt. Bei den Ribose- und Mannosederivaten, wurden α- und β-Formen als Kristalle isoliert. Eine neue stereospezifische Synthese von Xylofuranosylamino-imidazol über ein Xyloseoxazolin wird beschrieben.

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School of Chemistry, University of Bradford, Bradford B7 1DP (England), 29 November 1971.

[9] C. BRATTON and E. K. MARSHALL, J. biol. Chem. *128*, 537 (1939).
[10] For a discussion and further references on the use of NMR in the assignment of anomeric configurations, see L. GOLDMAN and J. W. MARSICO, J. med. Chem. *6*, 413 (1963).
[11] D. H. ROBINSON and G. SHAW, unpublished work.
[12] We wish to thank Professor W. KLYNE and Dr. P. M. SCOPES of Westfield College, Hampstead (England), for circular dichroism measurements.