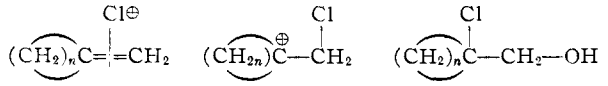
TABLE I

PRODUCT DISTRIBUTIONS IN HYPOHALOUS ACID ADDITIONS TO METHYLENECYCLOALKANES

Ring size	Hypochlorous acid: Total yield, %	Proportion of total, %: >C(Cl)CH₂OH	>C(OH)CH₂OH	Other product	Hypobromous acid: Total yield, %	Proportion of total, %: >C(Br)CH₂OH	Other products
C_4	72	60	40	none[a]	78	99	1[b]
C_5	64	41	58	1[b]	67	91	9[c]
C_6	92	67	32	1[b]	89	98	2[d]
C_7	86	35	64	1[b]	87	87	13[d]

[a] No rearrangement products were found. [b] Slight absorption attributable to a carbonyl group (probably in cycloalkanecarboxaldehyde) appeared in the infrared spectrum of the product. [c] Methylenecyclopentane oxide. [d] Cycloalkanecarboxaldehyde.

The preponderance of "abnormally" oriented products from the 4- and 6-membered rings is consistent with the view that trigonal carbons in those rings are unfavored.[6] The initially formed pi-complex (I) may rearrange (as usual) to the conventional carbonium ion (II),[3,7] or it may suffer direct attack by water to give III. Rearrangement of I to II is the less favored path for 4- and 6-membered rings, but is satisfactory for 5- and 7-membered



I II III

rings.[8] The formation of large amounts of III even from 5- and 7-membered rings, when acyclic olefins give corresponding products only in very small amounts, indicates that this explanation is incomplete and suggests an importance of the ring structure itself in directing orientation. That suggestion is further supported by the complete contrast between HOBr additions to methylenecycloalkanes and to isobutylene.

Since "abnormal" products were not obtained with HBr as addend,[2] it appears that the size of the attacking cation (or the "activity" of the attacking particle[9]) may have an important role in determining the actual orientation with these olefins. We hope that our continuing studies will reveal more explicit information about these effects.

Authentic 1-chloromethylcycloalkanols (except for 1-chloromethylcyclobutanol) were synthesized by hydrolysis of the appropriate methylenecycloalkane dichloride with an aqueous suspension of $CaCO_3$.[10] These chlorohydrins all reduced permanganate, gave precipitates with NaI in acetone and with alcoholic $AgNO_3$, produced immediate cloudiness in Lucas reagent and showed infrared absorption near 7.25 μ (tertiary alcohol). Authentic 1-halocycloalkylmethanols were synthesized by the action of hydrochloric or hydrobromic acid on the appropriate olefin oxide. These halohydrins all reduced permanganate, gave precipitates with alcoholic $AgNO_3$ but none with NaI in acetone, gave negative Lucas tests and showed no infrared absorption near 7.25 μ.

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(6) H. C. Brown, J. H. Brewster and H. Shechter, THIS JOURNAL, **76**, 467 (1954).

(7) Rearrangement of the protonated olefin (pi-complex) to a more conventional carbonium ion has been shown to be an important part of acid-catalyzed olefin hydration; R. W. Taft, Jr., *ibid.*, **74**, 5372 (1952); R. W. Taft, Jr., E. L. Purlee, P. Riesz and C. A. DeFazio, *ibid.*, **77**, 1584 (1955).

(8) A more complete discussion of this point is found in ref. 2.

(9) H. C. Brown and K. L. Nelson, THIS JOURNAL, **75**, 6292 (1953).

(10) C. E. Sparks and R. E. Nelson, *ibid.*, **58**, 1010 (1936).

URIDINE DIPHOSPHATE N-ACETYLGLUCOSAMINE AND URIDINE DIPHOSPHATE GLUCURONIC ACID IN MUNG BEAN SEEDLINGS

Sir:

Whereas the presence of uridine diphosphate N-acetylglucosamine (UDPaG) has been reported in penicillium, yeast and animal tissues[1-3] and uridine diphosphate glucuronic acid (UDPGuc) in liver tissue, these nucleotides have hitherto been unknown in higher plants.

In the present communication evidence is presented for the existence of UDPaG and UDPGuc in mung bean seedlings. Furthermore, these seedlings contain a hitherto unknown enzyme which catalyzes the reaction between UDPGuc and pyrophosphate to form uridine triphosphate (UTP) and presumably D-glucuronic acid 1-phosphate. While UDPGuc may be considered a possible precursor of the synthesis of polyuronides in plants, the function of the UDPaG is at present obscure.

The two nucleotides were isolated from 10 kg. of mung bean seedlings using methods described by Ginsburg, *et al.*[4] The nucleotide fraction eluted from the Dowex-1-Cl^- column by 0.01 *N* HCl–0.15 *N* NaCl was further purified by paper chromatography.[3] In this way 14 μmoles of a nucleotide (I) corresponding in R_f to authentic UDPaG (R_f 0.45) was separated. A second nucleotide fraction, eluted by 0.01 *N* HCl–0.06 *N* NaCl, was further purified by paper electrophoresis in formate buffer at *p*H 3.5[4,5] to yield 4 μmoles of a nucleotide (II) corresponding to UDPGuc in electrophoretic mobility.

Hydrolysis of both nucleotides with 0.1 *N* HCl at 100° for 10 minutes liberated chiefly uridine diphosphate, while hydrolysis with 1.0 *N* HCl yielded

(1) L. F. Leloir, Proc. 3rd Intern. Congr. Biochem., Brussels, 1955, p. 154.

(2) E. Cabib, L. F. Leloir and C. E. Cardini, *J. Biol. Chem.*, **203**, 1055 (1953).

(3) E. E. B. Smith and G. T. Mills, *Biochim. et biophys. Acta*, **13**, 386 (1954).

(4) V. Ginsburg, P. K. Stumpf and W. Z. Hassid, *J. Biol. Chem.*, **223**, 977 (1956).

(5) A. M. Crestfield and F. W. Allen, *Anal. Chem.*, **27**, 422, 424 (1955).

chiefly uridine monophosphate. In both cases a sugar was also set free. The degradation products were identified by paper chromatography and electrophoresis. Hydrolysis of I with 0.05 *N* HCl for 10 minutes at 100° yielded a substance which reacted as an N-acetylhexosamine.[6] It was identified as acetylglucosamine by paper chromatography,[2,7] and by paper electrophoresis at *p*H 5.8 (ammonium acetate) and *p*H 8.6 (borate).[8] Hydrolysis of I with 2 *N* HCl for 2 hours at 100° liberated a substance giving a positive reaction for hexosamine. This was identified as glucosamine by paper chromatography,[7,8] electrophoresis,[8] and by degradation to arabinose with ninhydrin.[7]

Mild acid hydrolysis of II set free a hexuronic acid (carbazole test) which was shown to be glucuronic acid by paper chromatography[9] and by electrophoresis at *p*H 3.5.[4,5] The analyses of the two nucleotides are presented in Table I. It is thus shown that substances I and II are UDPaG and UDPGuc, respectively.

TABLE I

ANALYSES OF NUCLEOTIDES FROM MUNG BEAN SEEDLINGS

	(I) μmole/μmole uridine[a]		(II)	
	Found	Calcd. for UDPaG	Found	Calcd. for UDPGuc
Total P[b]	2.10	2.00	2.02	2.00
Acid labile P[c]	0.98	1.00	0.95	1.00
Glucosamine[d]	0.97	1.00	..	..
Glucuronic acid[e]	..	..	0.93	1.00
Reducing value[f]	0	0	0	0
Reducing value after hydrolysis[g]	0.90	1.00	0.71	1.00

[a] Uridine moiety identified by characteristic ultraviolet spectrum at *p*H 2, 7 and 11. Concentration was determined by the optical density at 260 mμ (*p*H 7) calculated on the basis of a molar extinction of 9.9×10^3. [b] C. H. Fiske and Y. SubbaRow, *J. Biol. Chem.*, **66**, 375 (1925). [c] Hydrolysis with 0.1 *N* HCl for 10 minutes at 100°. [d] Z. Dische and E. Borenfreund, *J. Biol. Chem.*, **184**, 517 (1950). [e] Z. Dische, *ibid.*, **167**, 189 (1947). [f] J. T. Park and M. J. Johnson, *ibid.*, **181**, 149 (1949). [g] Hydrolysis with 0.1 *N* HCl for 10 minutes at 100°.

When UDPGuc (0.25 μmole) was incubated with a 55–65% ammonium sulfate fraction from a mung bean seedling extract in the presence of 0.5 μmole of sodium pyrophosphate, 2.5 μmoles of KF and 0.25 μmole of $MgCl_2$ in 0.02 *M* Tris buffer, *p*H 7.5, at 24° in a total volume of 17.5 μl. the formation of UTP could be demonstrated by electrophoresis at *p*H 3.5.[4,5] In analogy with other known UDP-sugar pyrophosphorylases,[10] the reaction may be formulated as

UDPGuc + pyrophosphate ⇌ UTP + glucuronic acid 1-phosphate

(6) D. Aminoff, W. T. J. Morgan and W. M. Watkins, *Biochem. J.*, **51**, 379 (1952).

(7) P. J. Stoffyn and R. W. Jeanloz, *Arch. Biochem. Biophys.*, **52**, 373 (1954).

(8) P. W. Kent and M. W. Whitehouse, "Biochemistry of the Aminosugars," Academic Press, Inc., New York, N. Y., 1955, p. 166.

(9) F. G. Fischer and H. Dörfel, *Z. physiol. Chem.*, **301**, 224 (1955).

(10) E. F. Neufeld, V. Ginsburg, E. W. Putnam, D. Fanshier and W. Z. Hassid, *Arch. Biochem. Biophys.*, in press.

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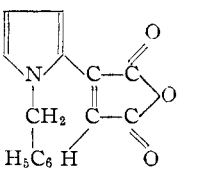
THE REACTION OF N-BENZYLPYRROLE WITH ACETYLENEDICARBOXYLIC ACID[1]

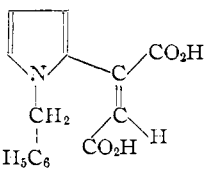
Sir:

The reaction of pyrroles with dienophiles has been shown to lead to substitution in the α-position of the pyrrole and, in some cases, to poly-substitution products such as dihydroindoles.[2] There has been no authenticated report of a pyrrole undergoing a normal Diels–Alder type of addition in the sense that, for example, furan will partake of Diels–Alder addition to dienophiles.[3]

We have found that refluxing an ether solution of N-benzylpyrrole and acetylenedicarboxylic acid for 24 hours gives rise to three products which were separated readily by taking advantage of their varying solubility properties. Two of these products, which had characteristic yellow colors, were shown to be α-substituted pyrroles on the basis of their elemental analyses, neutral equivalents, infrared spectra, common conversion by hydrolysis and hydrogenation to pyrrolidinesuccinic acid, and thus were assigned the structures I and II. This is in accord with the findings of Diels and Alder.[2]

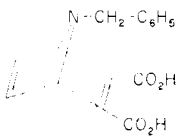


I



II

The third compound, which was colorless, was assigned structure III on the basis of the data given below.



III

The material had an elemental analysis (Calcd. for $C_{15}H_{13}O_4N$: C, 66.4; H, 4.8; N, 5.2. Found: C, 66.0; H, 4.9; N, 5.1.) and neutral equivalent (Calcd. for III: 135.5. Found: 133) which corresponded to a one-to-one adduct. The substance is amphoteric, being soluble in the cold in both dilute sodium bicarbonate and dilute hydrochloric acid, the latter indicative that the pyrrole moiety is no longer intact. On hydrogenation over 5% palladium on carbon at atmospheric pressure and room temperature, *three moles* of hydrogen were absorbed (in contrast to compounds I and II which take up *four moles* of hydrogen under these conditions) thus indicating the presence of a bicyclic skeleton. The material exhibited only end absorption in the ultraviolet (from 210 to 330 mμ) and its infrared spec-

(1) This work was supported by a Frederick Gardner Cottrell grant from Research Corporation.

(2) O. Diels and K. Alder, *Ann.*, **470**, 62 (1929); **486**, 211 (1931); **490**, 267 (1931); **498**, 1 (1932).

(3) H. Hopff and C. W. Rautenstrauch, U. S. Patent 2,262,002 [*C. A.*, **36**, 1046 (1942)] have reported without citing evidence, that N-isobutylmaleimide reacts with pyrrole to yield a tetrahydro-*endo*-phthalimide.