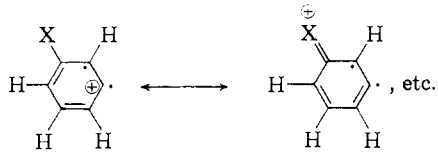
provided that the intermediate phenyl cation is regarded as in a state in which a π electron of the benzene ring has fallen with concerted uncoupling into

$$C_6H_5N_2^+ \xrightarrow{\text{r.d.}} C_6H_5^+ + N_2$$
$$\downarrow \text{fast}, +H_2O \longrightarrow C_6H_5OH + H^+$$

the open σ hybrid (sp^2) orbital of the cation (*i.e.*, the orbital occupied by the C–N sigma bond in $Ar-N_2^+$). This electronic rearrangement presumably gives a more stable structure with an odd number of π-electrons, in which this resonance can occur:



Since uncoupling to give a *triplet* phenyl cation seems necessary, the evidence cited herein appears to indicate that there are rapid rates of spin interconversions in the formation of the phenyl cation and in the reaction of this intermediate with the nucleophile, H_2O. The high reactivity of the intermediate has been established by Lewis[6] from its small degree of selectivity in reactions with nucleophiles.

The present argument is not inconsistent with the observation that –R *para* substituents strongly retard the rates of decomposition of the phenyl diazonium ion, provided that one regards (as previously[4,5]) the reaction transition state for such systems as being less stabilized than is the reactant phenyldiazonium ion.

The effects of reaction conditions[5] on "radical" and "cation" reaction products of the benzenediazonium decomposition, as well as heavy atom catalysis, are in keeping with the expected versatility of reaction of the proposed biradical carbonium ion. The possible role of ground state triplet cation formed in a primary process in many reactions of aromatic diazonium ions bears consideration.

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SEPARATION OF L-VALINE ACCEPTOR "SOLUBLE RIBONUCLEIC ACID" BY SPECIFIC REACTION WITH POLYACRYLIC ACID HYDRAZIDE

Sir:

To achieve the separation of amino acid-specific "soluble ribonucleic acids" (s-RNA), two basically different approaches have been applied in the past: the use of differences in physical properties of the various nucleotide chains[1–7] and the use of specific chemical reactions involving the ribose part of the adenylic acid end of the s-RNA[8–9] or the attached amino acid.[10] The principle of the separation we wish to describe is similar to that introduced by Zamecnik, Stephenson and Scott,[8] but offers the advantages of much higher yield at a high purification level. Moreover the time required is very short so that possible disruption of the secondary or tertiary structure of the s-RNA is probably minimized. In this procedure s-RNA is freed of all amino acids and then recharged with a single kind of amino acid. Only the remaining unlabelled s-RNA strands then remain available for a specific periodate oxidation to form a dialdehyde grouping on the end of the s-RNA. We found this "dialdehyde end" of the s-RNA to react readily with a specially prepared water-soluble polymer: polyacrylic acid hydrazide (PAAH) prepared according to the procedure of Kern, *et al.*[11] The oxidized s-RNA can be removed easily in this manner, leaving behind the single amino acid s-RNA in enriched form.

Yeast s-RNA prepared according to Monier, *et al.*,[12] was freed of amino acids by exposure to *p*H 10 at 37° for 30 minutes and relabelled with C^{14}-L-valine, using yeast enzyme.[3] Then 3 mg. of C^{14}-L-valine s-RNA were incubated for 5 minutes at room temperature with 0.1 *M* magnesium acetate buffer, *p*H 3.9, and 0.1 *M* $NaIO_4$ in a total volume of 1 ml. Most of the excess periodate was precipitated as the potassium salt by addition of 0.23 ml. 2 *M* KCl. After standing 5 minutes at 0° the KIO_4 was removed by centrifugation. The resulting solution of oxidized s-RNA was mixed with 4 ml. of polyacrylic acid hydrazide solution (containing 22.6 mg. of PAAH dissolved in 2 ml. of water and 2 ml. of 0.1 *M* magnesium acetate buffer *p*H 3.9) and thoroughly shaken at room temperature for 2 minutes. The PAAH s-RNA compound then was precipitated by reaction with 0.2 ml. (2.6 mM.) *n*-butyraldehyde with vigorous stirring and the precipitate was separated under suction on a sintered glass funnel. After removing unreacted traces of *n*-butyraldehyde by extraction with 20 ml. of ether and 10 ml. of ethyl acetate (two extractions with ether, then one with ethyl acetate and a fourth one with ether) the filtrate contained C^{14}-L-valine s-RNA in greatly enriched form. The ratio of absorbances at 280 and 260 mμ was the same before and after separation,

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indicating that the excess of PAAH has been removed by the *n*-butyraldehyde treatment. The specific activity increased from 1.15 mμM. C^{14}-L-valine/mg. s-RNA in the initial mixture to 11.5 mμM. C^{14}-L-valine/mg. s-RNA for the final solution (based on an assumed molecular weight for C^{14}-L-valine s-RNA of 25,000). This corresponds to a ten-fold enrichment and a purity of 28%. (For a molecular weight of 30,000 this would represent a purity of 34%. The recovery of the unoxidized s-RNA including the enriched C^{14}-L-valine s-RNA is essentially 100%, but that of the counts is 80%, indicative of some dissociation of the amino acid. The procedure described here is highly reproducible and may be used for larger quantities. With the high yield attained it is evident that repetitions of this procedure should lead to high purity of single amino-acid acceptor s-RNA in useful amounts.

Recent investigations have shown that PAAH also reacts with oxidized nucleosides and nucleotides, to form stable hydrazones. Neither cleavage of the phosphoester bond nor the release of the corresponding bases could be observed in nucleotides or s-RNA at acid or neutral *p*H, as reported by Khym and Cohn[13] and Saponara and Bock,[9] who used phenylhydrazine derivatives. Thus this polymer should find other uses in nucleic acid chemistry.

We wish to acknowledge generous help in the initial stages of this work from Dr. Yoshimi Kawade and useful discussions with Professor P. C. Zamecnik. H.v.P. gratefully acknowledges the support of a NATO Fellowship. This work was supported in part by National Cancer Institute Grant C-2170 and in part by AEC Contract At (30-1)-2643.[14]

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(14) This is publication No. 1048 of the Cancer Commission of Harvard University.

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CHEMISTRY OF CHALCOSE, A 3-METHOXY-4,6-DIDEOXYHEXOSE

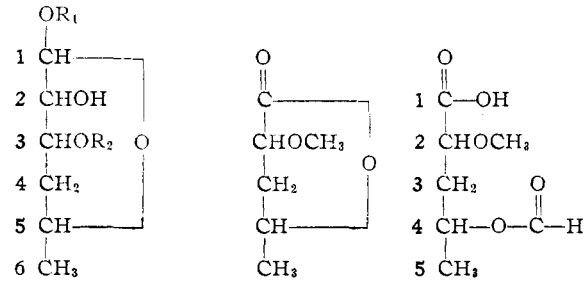
Sir:

Acid degradation of the antibiotic chalcomycin[1] has yielded chalcose (Ia), a 3-methoxy-4,6-dideoxyhexose. We wish to report the structural determination of this new sugar.

Methanolysis of chalcomycin yielded crystalline methyl chalcoside (Ib), m.p. 101.5–102°, $[\alpha]^{27}D$ −21° (*c* 2.04%, chloroform) [*Anal.* Calcd. for $C_8H_{16}O_4$: C, 54.53; H, 9.15; O, 36.32; C-CH_3 (1), 8.53; OCH_3 (2), 35.22. Found: C, 54.68; H, 9.25; O, 36.30; C-CH_3, 7.17; OCH_3, 33.7].

(1) Parke, Davis & Company, Belgian Patent 587,213, August 2, 1960.

Aqueous hydrolysis of methyl chalcoside gave crystalline chalcose (Ia), m.p. 96–99°, $[\alpha]^{24}D$ +120° (2 min.) → +97° (10 min.) → +76° (3 hr. and 26 hr.) (*c* 1.5%, water) [*Anal.* Calcd. for $C_7H_{14}O_4$: C, 51.84; H, 8.70; OCH_3 (1), 19.14. Found: C, 52.07; H, 8.93; OCH_3, 19.21], which could be reconverted to the crystalline methyl chalcoside by treatment with methanolic hydrogen chloride.



Ia, R_1 = H, R_2 = CH_3 II III
Ib, R_1 = R_2 = CH_3
Ic, R_1 = R_2 = H

Chalcose gave a positive Fehling test and a brown color with aniline hydrogen phthalate on papergrams. It reduced one mole of periodate, liberating no formaldehyde. Reduction of chalcose with sodium borohydride gave dihydrochalcose, which reduced one mole of periodate, liberating 0.7 mole of formaldehyde (chromotropic acid and dimedone methods). These data show that chalcose is a 2-hydroxy-aldosugar, as indicated by C_1 and C_2 of formula Ia.

Treatment of chalcose with fuming hydrobromic acid at 3° for 3 days[2] yielded de-O-methylchalcose (Ic), R_f 0.65 (chalcose R_f 0.70; *t*-butyl alcohol: acetic acid:water, 2:2:1). Treatment of Ic with two moles of periodate for 21 hours liberated (a), approximately one mole of acid (potentiometric titration), presumably formic acid from C_2 of formula Ic, and (b), crotonaldehyde (identified as the 2,4-dinitrophenylhydrazone), presumably formed by β-elimination of the oxygen function in 3-hydroxy(or 3-formyl)-butyraldehyde,[3] which originated from C_3 to C_6 of formula Ic. Both chalcose and dihydrochalcose gave slightly positive iodoform tests.

Oxidation of chalcose with periodate-permanganate[4] yielded a C-6 γ-lactone (II) (infrared absorption at 5.56 μ) and a crystalline C-7 acid (III), m.p. 68–69° [*Anal.* Calcd. for $C_7H_{12}O_5$ C, 47.72; H, 6.87; C-CH_3 (1), 8.53; OCH_3 (1), 17.62. Found: C, 48.00; H, 7.39; C-CH_3, 6.97; OCH_3, 16.99]. Treatment of the C-7 acid with 0.2 *N* sodium hydroxide resulted in the uptake of 2.02 equivalents of base, and gave after acidification, the same γ-lactone (II) mentioned earlier (identical infrared spectra). Reduction of the C-7 acid or the lactone with lithium aluminum hydride yielded an oily C-6 diol, characterized as its crystalline bis-3,5-dinitrobenzoate, m.p.

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