noted chain transfer agents and even the relatively strong H–Cl bond in hydrogen chloride is susceptible to free radical attack.[20]

(6) The isomerization of *p*-cymene (1-methyl-4-isopropylbenzene) exclusively to 1-methyl-4-*n*-propylbenzene is consistent with known free radical rearrangements[21] in that the relative positions of the two ring substituents are unchanged during the isomerization.

Experimental

Autoclave Experiments.—One to 10 mole % of an isomerization promoter (organic halides, mercaptans, etc.) and 0.1–0.5 mole of an alkylbenzene were placed in a 250-cc. autoclave.[22] After being sealed and flushed with nitrogen, the autoclave was rocked and heated at the desired temperature (300–525°) for 1 to 3 hours. The gaseous product was vented from the cooled vessel into a brine-displacement gas holder. The liquid product was washed with 20% sodium hydroxide solution, followed by water and dried over calcium chloride.

When reactions were carried out in sealed glass tubes, a calculated amount of the alkylbenzene was placed in the autoclave with the sealed tube. This procedure equalized the pressures inside and outside of the sealed tube, thereby preventing bursting of the latter at the elevated temperatures.

Flow Experiments.—The alkylbenzene and promoter were placed in a pressure-tight, stainless steel reservoir vessel mounted on a Metrogram balance. The reactants were forced, at a desired rate controlled by a needle valve, through a nitrogen-flushed line into a vertical reaction tube[22] (approximately 0.75 × 36 inches) held in an electrically heated furnace. This was accomplished by maintaining a nitrogen pressure inside the stainless steel reservoir vessel 100–250 p.s.i. greater than that in the reaction tube. The desired pressure in the reaction vessel (500–1500 p.s.i.) was maintained by a Grove regulator below the furnace. The residence times for the reactants in the vertical reactor were calculated without correction for non-ideality from the rates of decrease in weight of the reservoir vessel as measured by the Metrogram balance.

When desired, oxygen or inert gases were metered into the vertical reactor through a second line.

The liquid product was collected in an ice trap at the bottom of the vertical reactor and the gaseous product in a brine-displacement gas holder. The liquid product was washed and dried as above.

Product Analyses.—The gaseous and liquid products were analyzed both mass spectrometrically and by gas–liquid chromatographic techniques (g.l.c.) using a column packed with 30–40 mesh, silicone (DC-710)-impregnated firebrick. Compounds corresponding to the g.l.c. peaks were trapped and identified mass spectrometrically.

tert-Butylbenzene and isobutylbenzene in the products were determined by infrared analyzes. For this purpose, peaks at 13.10 and 13.55μ, respectively, were used. Infrared analysis was also employed for the detection of *o*-cymene, *m*-cymene and 1-methyl-3-*n*-propylbenzene resulting from the isomerization of *p*-cymene. These compounds apparently were not formed from *p*-cymene.

The following autoclave experiment is given as a typical example for the isomerization of cumene. At 450° and 750 p.s.i., using one mole % iodine promoter, 72% of the cumene was converted in 60 minutes. From 1.0 mole of cumene introduced, the following moles of hydrocarbons were recovered: CH_4, 0.067; C_2H_6, 0.043; C_3H_8, 0.014; C_4H_{10}, 0.006; $C_4H_8 + C_5H_{16}$, 0.0006; benzene, 0.040; toluene, 0.095; ethylbenzene, 0.143; unreacted cumene, 0.278; *n*-propylbenzene, 0.432; C_{10}–C_{18}, 0.001; solids (coke), 0.018.

Alkylbenzenes and Promoters.—The cumene, *tert*-butylbenzene, *sec*-butylbenzene, *n*-butylbenzene and *p*-cymene were of the highest purity available and were found by g.l.c. and infrared analyses to be essentially free of impurities. Whenever an impurity was detected, it was removed by efficient distillation of the alkylbenzene. The promoters were reagent grade materials and were further purified, where possible, by careful distillation.

Acknowledgments.—We wish to express our appreciation to P. A. Wadsworth and J. M. Gordon for mass spectrometric and infrared analyses and to L. F. Focha and G. W. Schoenthal for assistance in carrying out the autoclave and flow experiments.

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COMMUNICATIONS TO THE EDITOR

THE USE OF UREA TO ELIMINATE THE SECONDARY BINDING FORCES IN ION EXCHANGE CHROMATOGRAPHY OF POLYNUCLEOTIDES

Sir:

The separation of organic compounds by ion exchange chromatography is a complex process which is controlled, in part, by ionic interactions and by secondary binding forces. In the separation of proteins, these secondary forces are known to include hydrogen bonding and lipophilic interaction.[1] The latter force also has been shown to affect the separation of small molecules such as nucleotides and polynucleotides[2] and it now appears that hydrogen bonding could also be of importance. For example, studies on the chromatographic separation of thymidylic acid homopolymers[3] showed that polystyrene anion exchange resins strongly held all polymers larger than the dinucleotide, presumably because of the lipophilic interaction between the thymidine and polystyrene systems. This type of binding was circumvented by using a cellulose anion exchanger (DEAE-cellulose[4]) and a good separation of thymidylic acid polymers was thus achieved. However, attempts to separate mixed polynucleotides resulting from the enzymic degradation of deoxyribonucleic acids (DNA) (see Fig. 1A) and ribonucleic acids (RNA)[5] using the same technique

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(2) W. E. Cohn in E. Heftman, "Chromatography," Reinhold Publishing Corp., New York, N. Y., 1961, p. 554.

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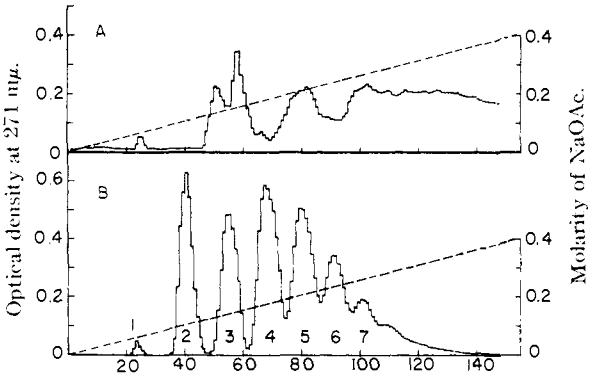

Fig. 1A.—Separation of components of a DNase I digest (200 O.D. units at 271 mμ) of salmon testes DNA: column, DEAE-cellulose-acetate, 20 × 1 cm., eluting solution, linear gradient of sodium acetate (pH 7.5) as shown (total volume, 1.5 l.). (B) Identical with (A) but with the eluting solution 7 M in urea.

have met with only limited success. We now wish to report that these mixed polynucleotides can be separated by a procedure which is dependent only on their degree of polymerization and independent of their base composition. The method employs DEAE-cellulose chromatography with the additional incorporation of urea, formamide or ethylene glycol into the eluting system.[6] This innovation eliminates, for practical purposes, the effects of secondary binding forces.

The separation of deoxyribopolynucleotides resulting from pancreatic deoxyribonuclease (DNase I) digestion of salmon testes DNA is shown in Fig. 1. In the absence of urea some separation of a dinucleotide was noted but the remainder of the digest was eluted in a broad band. However, in the presence of 7 M urea individual peaks emerged (numbered 1 to 7). These peaks were characterized as containing the mono-, di-, tri-, etc., nucleotides by their paper chromatographic and electrophoretic behavior and by determining the ratio of the phosphomonoesterase sensitive phosphate to the total phosphate in each. Additional confirmation was obtained by determining the ratio of nucleoside to nucleotide produced when a peak was treated with phosphomonoesterase followed by spleen or snake-venom phosphodiesterase.

The method is also applicable to ribopolynucleotide separation. The elution pattern obtained when a pancreatic ribonuclease digest of soluble ribonucleic acid (s-RNA) of yeast was fractionated is illustrated in Fig. 2.

This procedure offers a convenient method for initially fractionating polynucleotides. If this separation is followed by conventional chromatography on polystyrene or cellulose anion exchange resins extensive fractionation of the components of each peak would be expected. A detailed discussion of the above technique will be reported shortly along with applications of it to studies on the specificities of deoxyribonucleases and on the base sequences and end-groups of yeast s-RNA.

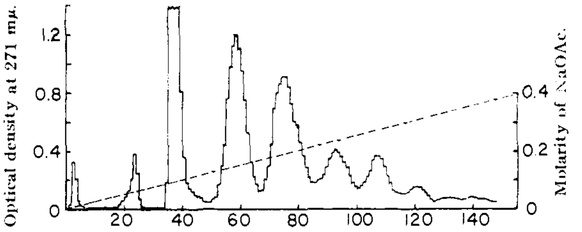

Fig. 2.—Separation of components of an RNase digest (550 O.D. units at 271 mμ) of yeast s-RNA: column, DEAE-cellulose-acetate, 20 × 1.8 cm.; eluting solution, 7 M in urea and a linear gradient of sodium acetate (pH 7.5) as shown (total volume 2 l.).

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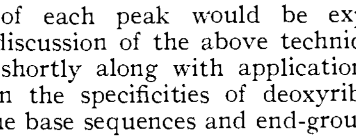
6-DEOXYTETRACYCLINES. III. STEREOCHEMISTRY AT C.6

Sir:

6-Deoxytetracyclines (Ia, Ib, Ic) have been prepared earlier[1,2] through catalytic hydrogenolysis of the benzyl hydroxyl group in tetracycline, oxytetracyline, or 6-demethyltetracycline (IIa, IIb, or IIc).[3] These acid stable analogs have proven to be key substances in delineating qualitative structure-activity relationships in the tetracycline series—both from their own biological properties[1,2] and from studies on their aromatic substitution



Ia $R_1 = CH_3$, $R_2 = R_3 = H$
Ib $R_1 = CH_3$, $R_2 = H$, $R_3 = OH$
Ic $R_1 = R_2 = R_3 = H$
IIa $R_1 = CH_3$, $R_2 = OH$, $R_3 = H$
IIb $R_1 = CH_3$, $R_2 = R_3 = OH$
IIc $R_1 = R_3 = H$, $R_2 = OH$
IId $R_1, R_2 = CH_2$, $R_3 = H$
IIe $R_1, R_2 = CH_2$, $R_3 = OH$

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