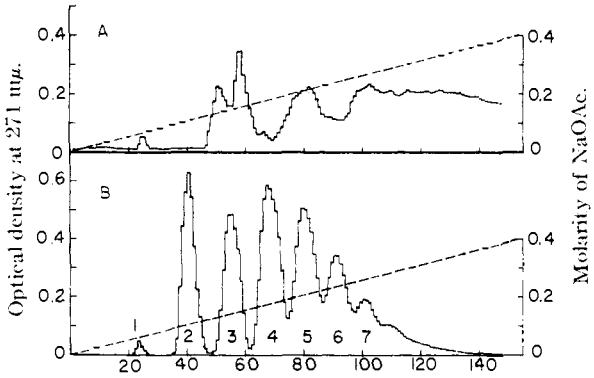

Fig. 1A.—Separation of components of a DNase I digest (200 O.D. units at 271 mμ) of salmon testes DNA: column, DEAE-cellulose-acetate, 20 × 1 cm., eluting solution, linear gradient of sodium acetate (pH 7.5) as shown (total volume, 1.5 l.). (B) Identical with (A) but with the eluting solution 7 *M* in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

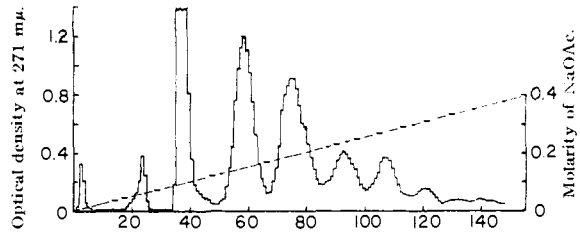

Fig. 2.—Separation of components of an RNase digest (550 O.D. units at 271 mμ) of yeast *s*-RNA: column, DEAE-cellulose-acetate, 20 × 1.8 cm.; eluting solution, 7 *M* in urea and a linear gradient of sodium acetate (pH 7.5) as shown (total volume 2 l.).

This work was supported by the National Institutes of Health, U. S. Public Health Service grant C-5342.

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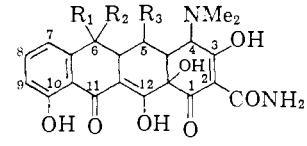
RECEIVED MAY 16, 1962

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



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, $R_2 = CH_2$, $R_3 = OH$

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

(5) M. Staehelin, *Biochem. Biophys. Acta*, **49**, 11 (1961); M. Staehelin, H. A. Sober and E. A. Peterson, *Arch. Biochem. Biophys.*, **85**, 289 (1959).

(6) Chromatographic systems containing urea have been used in the purification of proteins[7] but, as far as we are aware, this is the first report of their use in overcoming secondary binding forces between small polymers and cellulose ion exchangers.

(7) R. D. Cole, *J. Biol. Chem.*, **235**, 2294, 2300 (1960); E. O. P. Thompson and I. J. O'Donnell, *Australian J. Biol. Sci.*, **13**, 393 (1960).

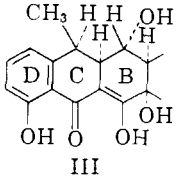
(1) C. R. Stephens, K. Murai, H. H. Rennhard, L. H. Conover and K. J. Brunings, *J. Am. Chem. Soc.*, **80**, 5324 (1958).

(2) J. R. D. McCormick, E. R. Jensen, P. A. Miller and A. P. Doerschuk, *ibid.*, **82**, 3381 (1960).

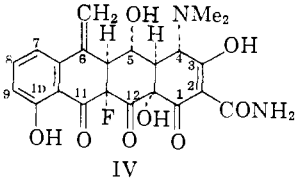
(3) (a) F. A. Hochstein, C. R. Stephens, L. H. Conover, P. P. Regna, R. Pasternack, P. N. Gordon, F. J. Pilgrim, K. J. Brunings and R. B. Woodward, *ibid.*, **75**, 5455 (1953). (b) C. R. Stephens, L. H. Conover, R. Pasternack, F. A. Hochstein, W. T. Moreland, P. P. Regna, F. J. Pilgrim, K. J. Brunings and R. B. Woodward, *ibid.*, **76**, 3568 (1954). (c) J. R. D. McCormick, M. O. Sjolander, V. Hirsch, E. R. Jensen and A. P. Doerschuk, *ibid.*, **79**, 4561 (1957).

products.[4,5] Total synthesis[6] efforts on 6-deoxytetracyclines and related degradation products also have posed interesting chemical and stereochemical problems. As is apparent, certain 6-deoxy analogs, *e.g.*, Ia, Ib, contain an asymmetric center (C.6) which *may* or *may not* have the same configuration as that present in the parent 6-hydroxy compounds (IIa, IIb). No experimental evidence bearing on this question has appeared although Muxfeldt[7] has suggested from synthesis studies that inversion of the C.6 methyl may have occurred in the hydrogenolysis of 7-chlorotetracycline. This paper records the preparation of *both* C.6 epimers of Ia and Ib and the establishment of their configurations relative to the parent 6-hydroxy compounds. Our results confirm the conclusion that C.6 inversion had occurred in the earlier 6-deoxy compounds Ia and Ib.

Hydrogenation of 6-methylene-5-hydroxytetracycline[8] (IIe) over a noble metal catalyst yields, as the predominant product, a 1:1 mixture of the C.6 epimers of Ib. These may be separated readily by chromatographic procedures or by fractional crystallization. The epimer with lowest biological activity, which we now call β-6-deoxy-5-hydroxytetracycline, is identical with the previously reported "6-deoxyoxytetracycline.[1]" α-6-Deoxy-5-hydroxytetracycline, ($[\alpha]^{25}D$ −109° (*c* 1, 0.01 *N* HCl in methanol); λ_{max} 267, 351 mμ, log ε 4.24, 4.12 (0.01 *N* HCl in methanol). Calcd. for $C_{22}H_{24}N_2O_8 \cdot CH_3OH \cdot HCl$: C, 53.86; H, 5.70; N, 5.46; Cl, 6.91. Found: C, 53.96; H, 5.73; N, 5.30; Cl, 6.84) shows biological activity (both *in vitro* (Table I) and *in vivo*[9] (in mice)) which is substantially greater than that of the earlier epimer. The ultraviolet absorption of α-6-deoxy-5-hydroxytetracycline more closely resembles that of oxytetracycline than that of the β-epimer. Chemical information on the configuration of the C.6 methyl in the β series (*cf.* expression III)[10] was forthcoming when we observed that catalytic hydrogenation of 11a-fluoro-6-methylene-5-hydroxytetracycline[11] IV (λ_{max} MeOH/0.01 *N* HCl 237, 268, 366 mμ, log ε 4.25, 4.23, 3.48; λ_{max}^{KBr} 5.7 μ) yields predominantly



III



IV

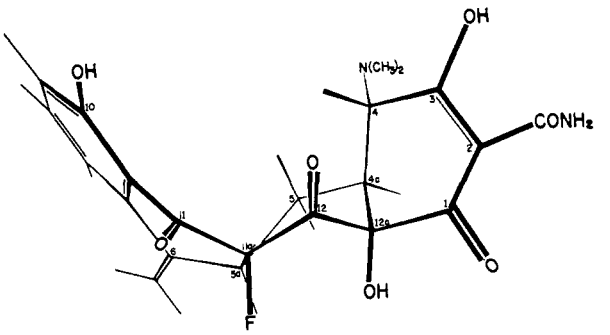
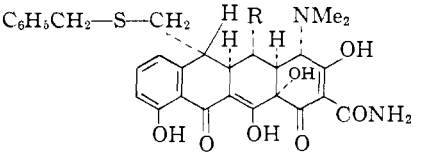


Fig. 1.

the β-epimer. The skeletal configuration of IV is established.[10,12] Model studies indicate clearly that IV is best represented by the conformation shown in Fig. 1. It can be seen that the curvature of the molecule is such that the methylene function can approach the catalyst most readily from the side of the C.5a hydrogen. Attack of hydrogen in this manner would result in the β-configuration at C.6, with the C.6 methyl and C.5a hydrogen *trans*.

Further confirmation of the C.6 stereochemistry was obtained by an alternative, stereospecific synthesis of the α-epimer of Ib. It has been observed that benzylmercaptan will add to the methylene group of IIe through a free radical mechanism to give the adduct Va.[13] Only one C.6 epimer of Va is obtained. From model studies we would



Va, R = OH
Vb, R = H

expect the more stable form to be that with the large group at C.6 equatorial, or with the α-configuration as shown. Raney nickel desulfurization of Va leads exclusively to the α-6-deoxy-5-hydroxytetracycline (Ib).

Catalytic hydrogenation of 6-methylenetetracycline[8] (IId) results in a substantial yield of the previously reported "6-deoxytetracycline"[1] together with a smaller quantity of a new, more highly active epimer. Considerable amounts of anhydrotetracycline[14] and its reduction products[1,2] also are formed in this reaction. Application of the mercaptan addition–Raney nickel desulfurization procedure to 6-methylenetetracycline yields the new epimer, α-6-deoxytetracycline (calcd. for $C_{22}H_{24}N_2O_7 \cdot HO_3SC_7H_7$: C, 57.99; H, 5.37; N, 4.66. Found: C, 57.73; H, 5.43; N, 4.62; λ_{max} 269, 352 mμ; log ε 4.28, 4.2 (0.01 *N* HCl in methanol)) as the only isolated product. Configurational

(4) J. J. Beereboom, J. J. Ursprung, H. H. Rennhard and C. R. Stephens, *ibid.*, **82**, 1003 (1960).

(5) J. J. Hlavka, A. Schneller, H. Krazinski and J. H. Boothe, *ibid.*, **84**, 1426 (1962).

(6) *Cf.* H. Muxfeldt, W. Rogalski and K. Strigler, *Angew. Chem.*, **72**, 170 (1960); T. L. Fields, A. S. Kende and J. H. Boothe, *J. Am. Chem. Soc.*, **82**, 1250 (1960); *ibid.*, **83**, 4612 (1961).

(7) H. Muxfeldt, private communication.

(8) R. K. Blackwood, J. J. Beereboom, H. H. Rennhard, M. Schach von Wittenau and C. R. Stephens, *ibid.*, **83**, 2773 (1961).

(9) We are indebted to Dr. A. R. English and to Dr. T. M. McBride for permission to disclose their unpublished animal results.

(10) Stereochemical representations herein are based on the X-ray data of Takeuchi and Buerger, *Proc. Natl. Acad. Sci.*, **46**, 1366 (1960). Chemical data (*cf.* ref. 12 and footnote 41 of ref. 3a) confirm the skeletal arrangement at the centers pertinent to the present argument—*e.g.*, C.4a, C.12a, C.5a and C.11a in Fig. 1.

(11) Prepared by methods previously outline by Blackwood *et al.* (*cf.* ref. 7) from 11α-fluoro-5-hydroxytetracycline-6,12-hemiketal (ref. 12).

(12) H. H. Rennhard, R. K. Blackwood and C. R. Stephens, *J. Am. Chem. Soc.*, **83**, 2774 (1961).

(13) R. K. Blackwood, *et al.*, in press.

(14) C. W. Waller, B. L. Hutchings, R. W. Broschard, A. A. Goldman, W. T. Stein, C. F. Wolf and T. H. Williams, *J. Am. Chem. Soc.*, **74**, 4981 (1952).

assignment of this rests on analogy to the previous case.

In vitro biological data on the 6-deoxy epimers (Ia, Ib) as well as on known 6-deoxy-6-demethyltetracycline[1,2,4] (Ic, a compound with no asymmetry at C.6) are presented in Table I.[15] The

TABLE I

Compound	Bioassay[a] *vs. K. Pneumoniae*[b]
6-Dimethyl-6-deoxytetracycline (Ic)	900
α-6-Deoxytetracycline (Ia)	700
α-6-Deoxy-5-hydroxytetracycline (Ib)	1400
β-6-Deoxytetracycline (Ia)	500
β-6-Deoxy-5-hydroxytetracycline (Ib)	400

[a] Expressed in oxytetracycline units/mg. *Cf.* R. C. Kersey, *J. Am. Pharm. Assocn.*, **39**, 252 (1950). In this assay 5-hydroxytetracycline is taken as the standard at 1000 units/mg. [b] Similar relative activities have been noted with other microörganisms.

reduced activity in the β-series may well be the result of conformational distortion.

(15) We are indebted to Mr. J. J. Smith and his associates for these data.

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RECEIVED MAY 24, 1962

HOT RADICAL EFFECTS IN AN INTRAMOLECULAR INSERTION REACTION

Sir:

Thermal decomposition of *p*-toluenesulfonylhydrazones of aldehydes and ketones in basic media gives rise to diazocompounds, which in aprotic solvents themselves undergo decomposition by a carbenoid process.[1] The decomposition of the tosylhydrazone of 2-butanone[2] in diethylcarbitol in the presence of sodium methoxide yields *cis*- and *trans*-butene-2, butene-1 and a trace of methylcyclopropane (see table). These compounds presumably are formed from methylethylcarbene by reactions that can be considered as intramolecular insertion reactions formally similar to the intermolecular insertion reactions of methylene itself.[3]

We wish to report that the thermal decomposition of methylethyldiazirine in the gas phase at ~ 160° also results in the formation of these hydrocarbons in ratios virtually identical with those reported by Friedman and Shechter.[2] There can be little doubt that this reaction proceeds *via* the formation of the carbene which supports a similar mechanism for the decomposition of the tosylhydrazones. Photolysis of methylethyldiazirine (3130 Å. radiation) also results in the formation of the same C_4H_8 hydrocarbons but in quite different ratios.

In the photolyses the relative yields of the products were independent of the pressure of the diazirine in the range 50 to 200 mm. To eliminate the possibility of secondary isomerization of the initially formed excited olefins, photolyses were carried out in the presence of added nitrogen, at pressures up to 2 atmospheres, without changing the ratios of the products. Thus the compositions shown in the table represent the *initial* rearrangement ratios from the photolytically produced carbene. Below 50 mm. some small variations in the product ratios were observed, which could be rationalized in terms of the secondary isomerization of the methylcyclopropane (initially formed with excess energy) to butenes. At 4 mm. the yield of methylcyclopropane had fallen to 1.0%, the yield of *trans*-butene-2 had risen to 42% and a trace of isobutene was detected.

TABLE I

% Composition	1[a]	2[b]	3[c]
Butene-1	5	3.6	23.4
trans-Butene-2	67	66.5	38.6
cis-Butene-2	28	29.5	35.6
Methylcyclopropane	0.5	0.4	2.4

[a] Friedman and Shechter (ref. 2). [b] This work, pyrolysis at ~160°. [c] This work, photolysis (3130 Å.).

The relatively high proportion of butene-1 and methylcyclopropane, and the near equivalence of the amounts of *cis*- and *trans*-butene-2 in the photolytic decomposition are in striking contrast to those from the pyrolyses. These differences are most reasonably explained by postulating that the carbene produced photochemically is vibrationally excited. The results, therefore, indicate a hot radical effect in intramolecular insertion reactions of carbenes previously only noted in intermolecular reactions.[4]

(1) J. W. Powell and M. C. Whiting, *Tetrahedron*, **7**, 305 (1959).

(2) L. Friedman and H. Shechter, *J. Am. Chem. Soc.*, **81**, 5512 (1959).

(3) W. E. Doering, R. G. Buttery, R. G. Laughlin and N. Chaudhuri, *ibid.*, **78**, 3224 (1956).

(4) H. M. Frey, *ibid.*, **80**, 5005 (1958).

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RECEIVED APRIL 14, 1962

AN ISOTOPE EFFECT DURING THE COUNTERCURRENT DISTRIBUTION OF ARABINOSE-1-C^{14}

Sir:

Insofar as we know, the literature lacks any reference to an isotope effect influenced by the position of C^{14} in a sugar—or other solute—undergoing countercurrent distribution. For this reason, we wish to report the data below.

D-Arabinose-1-C^{14} (K = 0.11) moved more slowly than unlabeled D-arabinose (Fig. 1) during countercurrent distribution in cyclohexane–95% ethanol with the result that specific activity decreased with increasing number of the tubes of the train constituting the sugar zone. D-Arabinose-1-C^{14} and unlabeled L-arabinose also were partly resolved (Fig. 2); similarly L-arabinose-1-C^{14} and unlabeled D-arabinose were partly separated. In all cases, such plots of log specific activity against fraction number for tubes containing this pentose were linear and were never parallel to the abscissa, an index of resolution.[1] When radioactivity resided on carbon 5, the mobilities of the radioactive and unlabeled D-arabinose were indistinguishable, after 350 or 800 transfers. Neither D-xylose-1-C^{14} (K = 0.26) nor D-ribose-1-C^{14} (K

(1) K. A. Piez and H. Eagle, *J. Am. Chem. Soc.*, **78**, 5284 (1956).