almost completely dissolved in 75 cc. of reagent chloroform, and 2.124 g. of N-bromosuccinimide (0.012 mole) was added. Within a few minutes a heavy but flocculent precipitate formed which, with continuous stirring, returned almost completely into solution. After clarification the reaction filtrate yielded the dibromo compound after standing at room temperature overnight. The almost white crystalline product was filtered, washed with ether and dried at 60° *in vacuo;* wt. 1.39 g. The filtrate yielded a second crop after further standing which was isolated in the same manner; wt. 153 mg., total yield 53.3%. This compound crystallizes either from 2-methoxyethanol and 0.1 *N* hydrochloric acid or from glacial acetic acid in the same way as did compound II; m p. 250° with dec. The ultraviolet spectra show $\lambda_{max}^{0.1N\ HCl}$ 255–275 mμ (flat max.) (log ε 4.174), 350 mμ (log ε 3.579); $\lambda_{max}^{0.1M\ Na_2B_4O_7}$ 240 mμ (log ε 4.598); 285 mμ (log ε 4.132); 315, 330, 345 mμ (log ε 3.978).

Anal. Calcd. for $C_{20}H_{17}NO_7Br_2$: C, 44.2; H, 3.13; N, 2.57; Br, 29.4. Found: C, 44.45; H, 3.46; N, 2.58; Br, 28.51.

Dibromo-12a-deoxydedimethylaminoanhydrotetracycline (X).—(a) A solution of 1.468 g. (0.004 *M*) of anhydro-12a-deoxydedimethylaminotetracycline (VI) and 1.416 g. (0.008 *M*) of N-bromosuccinimide at room temperature deposited crystalline precipitate within 1.5 hours. The compound was isolated and dried for 3 days without heat in vacuum; wt. 1.2 g., yield 46.5%. This orange-red compound contains one mole of chloroform of crystallization which is lost upon recrystallization from methanol. The ultraviolet spectra show $\lambda_{max}^{0.1N\ HCl}$ 228 mμ (log ε 4.544), 277 mμ (log ε 4.615), 440 mμ (log ε 3.903); $\lambda_{max}^{0.1M\ Na_2B_4O_7}$ 232 mμ (log ε 4.458), 275 mμ (log ε 4.538), 440 mμ (log ε 4.021).

Anal. Calcd. for $C_{20}H_{17}NO_6Br_2$: C, 45.6; H, 2.86; N, 2.69; Br, 30.5. Found: C, 45.55; H, 3.02; N, 2.49; Br, 29.67.

(b) A solution of 250 mg. of 11a,12a-dibromo-12a-deoxydedimethylaminotetracycline (VIII) in 15 cc. of hot acetic acid was cooled and 30 mg. of the starting material crystallized and was filtered off. To the filtrate was added 0.5 cc. of 31% hydrobromic acid in acetic acid, and after heating on the steam-bath for some time a small amount of solid precipitate formed. After filtering off the solid (30 mg.), the filtrate was diluted with 35 cc. of water. The orange precipitate was filtered off and dried; wt. 70 mg. This product was shown by spectral evidence to be identical with that described under (a).

Bromodedimethylaminoanhydro-7-chlorotetracycline.—To a solution of 9.44 g. of dedimethylamino-7-chlorotetracycline[2] in 50 ml. of dimethoxyethane was added 100 ml. of glacial acetic acid and 11.05 ml. of bromine at room temperature. Orange crystals rapidly deposited and after standing for 4 hours the mixture was heated on the steam-bath for 1 hour and cooled. The orange hexagonal plates were filtered and dried to yield 8.54 g., m.p. 250–260° dec. After one recrystallization from dimethylformamide and methanol the m.p. remained the same. The ultraviolet spectra show $\lambda_{max}^{0.1N\ HCl}$ 232 mμ (log ε 4.448), 276 mμ (log ε 4.527), 440 mμ (log ε 4.527); $\lambda_{max}^{0.1M\ Na_2B_4O_7}$ 237 mμ (log ε 4.545), 273 mμ (log ε 4.638), 350 mμ (log ε 3.829), 444 mμ (log ε 4.110).

Anal. Calcd. for $C_{20}H_{15}NO_7BrCl$: C, 48.3; H, 3.04; N, 2.82; Cl, 7.14. Found: C, 48.20; H, 3.34; N, 2.99; Cl, 7.59.

Bromodedimethylaminoiso-7-chlorotetracycline.—To a cooled solution of 0.94 g. (0.002 *M*) of dedimethylamino-7-chlorotetracycline dissolved in 10 ml. of dimethoxyethane and 10 ml. of acetic acid was added 0.69 g. (0.00216 *M*) of pyridinium bromide perbromide dissolved in 10 ml. of dimethoxyethane. The solution stood in an ice-bath 10 minutes when 0.95 ml. of 25% aqueous sodium acetate (0.0023 *M*) was added. This solution stood at room temperature for 40 minutes after which some of the solvent was removed under an air-jet. Upon adding 80 ml. of water a flocculent yellow precipitate was obtained. This was filtered, washed well with water and dried at 60° for 1 hour *in vacuo;* wt. 0.85 g. The ultraviolet spectra show $\lambda_{max}^{0.1N\ HCl}$ 220 mμ (log ε 4.369), 247 mμ (log ε 4.210), 263 mμ (log ε 4.218), 312 mμ (log ε 3.573), 365 mμ (log ε 3.255); $\lambda_{max}^{0.1M\ Na_2B_4O_7}$ 223 mμ (log ε 4.366), 256 mμ (log ε 4.151), 281 mμ (log ε 4.230), 344 mμ (log ε 3.754). The infrared spectrum showed the peak at 5.72 μ characteristic of the phthalide carbonyl of isotetracyclines.

Anal. Calcd. for $C_{20}H_{17}O_8NClBr$: N, 2.72; Cl, 6.89. Found: N, 2.75; Cl, 6.92.

Dibromodedimethylaminoisochlorotetracycline.—A suspension of 888 mg. (0.0025 *M*) of dedimethylamino-7-chlorotetracycline in 25 cc. of chloroform was treated with 404 mg. of N-bromosuccinimide (0.0025 *M*) in 5 cc. of chloroform. The complete solution which formed was permitted to stand at room temperature overnight during which time a crystalline product deposited. The reaction flask was cooled for 2 days and the almost white precipitate was filtered, washed with chloroform and ether, and then dried in vacuum at 60°; wt. 380 mg., yield 36%. The compound was recrystallized first from 15 cc. of acetic acid and 30 cc. of water, and then from 15 cc. of acetic acid and 80 cc. of water; wt. 180 mg. The compound may also be crystallized from 2-methoxyethanol and water; m.p. 205–210° dec. The ultraviolet spectra show $\lambda_{max}^{0.1N\ HCl}$ 220 mμ (log ε 4.494), 241 mμ (log ε 4.285), 260–263 mμ (shoulder) (log ε 4.171), 315 mμ (log ε 3.774); $\lambda_{max}^{0.1M\ Na_2B_4O_7}$ 228 mμ (log ε 4.345), 260 mμ (log ε 4.171), 284 mμ (log ε 4.228), 350 mμ (log ε 3.834).

Anal. Calcd. for $C_{20}H_{16}NO_8ClBr_2$: C, 40.4; H, 2.69; N, 2.36. Found: C, 40.32; H, 2.81; N, 2.26.

[CONTRIBUTION FROM THE ORGANIC CHEMISTRY RESEARCH SECTION, LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID CO., PEARL RIVER, N. Y.]

Chemistry of the Tetracycline Antibiotics. III. 12a-Deoxytetracycline

BY ARTHUR GREEN AND JAMES H. BOOTHE

RECEIVED NOVEMBER 4, 1959

The preparation of a 12a-deoxytetracycline by means of a reduction with zinc and ammonium hydroxide is described. The proof of structure by means of spectral data and chemical transformations is discussed. The conversion of 12a-deoxytetracycline to a known oxytetracycline[1] degradation product, dedimethylaminoterrarubein, is described.

Part of our work on the chemistry of the cycline antibiotics has been designed to attempt the selective removal of single functional groups or atoms without affecting the remainder of the molecule. The purpose of such work was to determine which of the various functional groups are necessary for the biological activity of these antibiotics. A number of examples of tetracyclines which differ from each other by only one group or atom are known, prepared either by means of chemical transformations or fermentations. In addition to the well known tetracycline, chlorotetracycline and oxytetracycline,[1] mutant strains of *Streptomyces aureo-*

(1) The trademark of American Cyanamid Co. for tetracycline is Achromycin and the trademark of Chas. Pfizer and Co., Inc., for this compound is Tetracyn. The trademark of American Cyanamid Co. for chlorotetracycline is Aureomycin. The trademark of Chas. Pfizer and Co., Inc., for oxytetracycline is Terramycin.

faciens have produced 6-demethyltetracycline and 6-demethylchlorotetracycline.[2] The dimethylamino group has been selectively removed from several of the tetracyclines by reduction of either the parent tetracycline[3,4] or its quaternary methiodide[5] with zinc and acetic acid. Recently several additional examples of the selective removal of single functional groups were added to the literature with the description of a number of 6-deoxytetracyclines.[6]

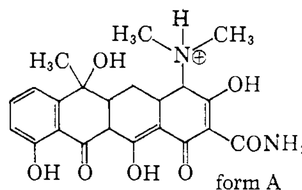
In this paper we are reporting the preparation of yet another example of the selective removal of a functional group, namely, the tertiary hydroxyl group in the 12a-position. It was found that treatment of tetracycline with zinc dust and 15% aqueous ammonium hydroxide yielded a mixture of products from which 12a-deoxytetracycline could be isolated in yields as high as 40%. The compound exists as bright red crystals in the solid state, but in solution exhibits a yellow color with strong fluorescence. 12α-Deoxytetracycline has a low order of antibacterial activity showing only about 2% of the activity of tetracycline when tested *in vitro* against *Staph. aureus*.

The structure of this compound was deduced from its empirical formula and ultraviolet absorption spectra, and was proved unequivocally by its chemical transformations. The ultraviolet absorption spectra are extremely complex, the length of the chromophore depending upon the degree and direction of enolization of the polycarbonyl system which, because of the removal of the blocking group at position 12a, can now exist as one completely conjugated chromophore. However, a comparison of the spectra of tetracycline and 12α-deoxytetracycline taken in 0.1 *N* hydrochloric acid shows the 12a-deoxy derivative to have a shorter chromophore. This also was observed with the 12a-deoxydedimethylaminoöxytetracycline[4] and the corresponding chlorotetracycline derivative,[3] and was ascribed to an alternate enolization of the 12-carbonyl group. However, when the spectra of 12a-deoxytetracycline were taken in other media, absorption maxima at much longer wave lengths were noted, indicating that other tautomeric forms were contributing to a longer chromophore. Similar long wave length absorptions also were observed with 12a-deoxydedimethylaminotetracycline.[7a] The ultraviolet absorption spectra of 12a-deoxytetracycline in several different media are summarized in Table I. Our suggested interpretation of the tautomeric forms which contribute most to the major maxima above 300 mμ are also included, although it must be recognized that any of the various spectra may represent several tautomeric forms in equilibrium.

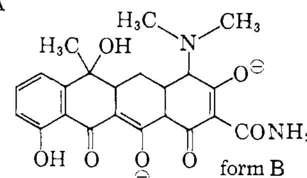
Table I

Ultraviolet Absorption of 12a-Deoxytetracycline

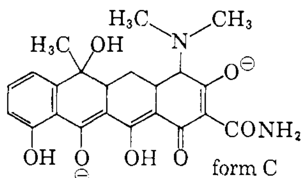
Medium	λ_{max}	log ϵ	Enolic form
0.1 *N* HCl	265	4.322	
	325	4.164	A
0.1 *M* sodium borate	265	4.110	
	380–400	4.047	B
	465	4.255	C
Methanol	260	4.200	
	330	4.030	A
	455 and 480	4.064 av.	D
10 cc. of methanol plus 1 drop of 0.1 *N* NaOH	368	3.912	
	470 and 495	4.332 av.	D



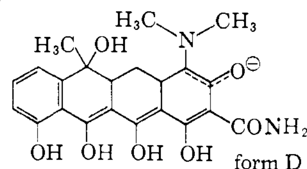
form A



form B



form C



form D

Chemical transformations which established unequivocally the structure of 12a-deoxytetracycline included its facile dehydration with mineral acid to form anhydro-12a-deoxytetracycline (IV), which showed that there had been no reduction of the 6-hydroxyl group. 12a-Deoxytetracycline also was reduced with zinc and dilute acetic acid to the corresponding 12a-deoxydedimethylaminotetracycline,[3,7a] which had been independently prepared directly from tetracycline. For purposes of comparison, the compound was dehydrated by treatment with strong acid to the known anhydro-12a-deoxydedimethylaminotetracycline[7a] which is more amenable to purification by recrystallization.

In an attempt to rehydroxylate the 12a-position of 12a-deoxytetracycline[7b] (II) with perbenzoic acid, an unexpected result was observed. The only crys-

(2) J. R. McCormick, N. O. Sjolander, U. Hirsch, E. R. Jensen and A. P. Doerschuk, This Journal, **79**, 4561 (1957).

(3) 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).

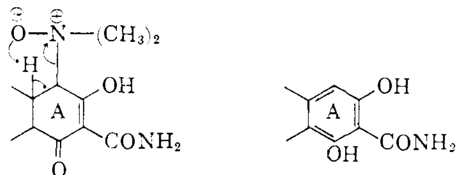
(4) F. A. Hochstein, C. R. Stephens, L. H. Conover, P. P. Regna, R. Pasternack, P. N. Gordon, F. J. Pilgrim, K. J. Brunings and R. B. Woodward, *ibid.*, **75**, 5455 (1953).

(5) J. H. Boothe, G. E. Bonvicino, C. W. Waller, J. P. Petisi, R. G. Wilkinson and R. B. Broschard, *ibid.*, **80**, 1654 (1958).

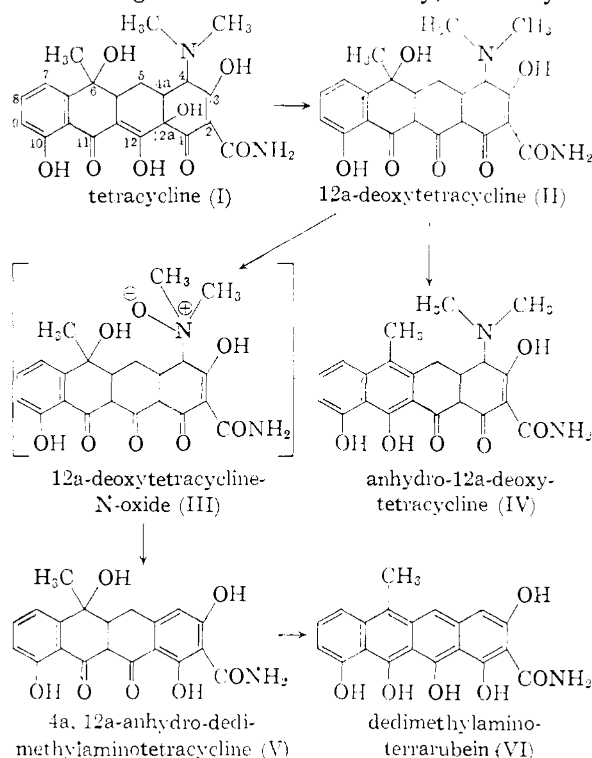
(6) C. R. Stephens, R. Murai, H. H. Rennhard, L. H. Conover and K. J. Brunings, *ibid.*, **80**, 5324 (1958).

(7) (a) Arthur Green, R. G. Wilkinson and J. H. Boothe, This Journal, **82**, 3946 (1960). (b) Recently another group of workers from these laboratories have reported on the successful hydroxylation of 12a-deoxytetracycline by two different methods; see C. E. Holmlund, W. W. Andres and A. J. Shay, This Journal, **81**, 4748, 4750 1959). Added in Proof.—(c) The use of acidified methanol as a solvent for determining ultraviolet absorption spectra of 12a-deoxytetracycline should be avoided since it has been observed by Mr. E. Jensen that a rather rapid change to the corresponding anhydro compound occurs in 0.1 *N* methanolic HCl.

talline compound which could be isolated from this reaction mixture was identified as 4a,12a-anhydrodedimethylaminotetracycline (V), isolated in about 20% yield. This substance had previously been obtained through an independent series of reactions[7a] and thus was identified readily by comparison of analytical and ultraviolet absorption data. Its formation from 12a-deoxytetracycline may be rationalized in terms of an intermediate N-oxide which can decompose by loss of N,N-dimethylhydroxylamine to give the observed aromatic product. Such elimination with olefin formation is well known and is proposed to occur through a *quasi*-5-membered ring transition state involving a *cis*-hydrogen in the β-position.[8]



The relative stereochemistry of the dimethylamino group at position 4 and the hydrogen at position 4a has been mentioned at least twice in the literature. Although one source suggests a *trans* relationship[4] while the other favors a *cis* configuration,[9] neither presents experimental evidence in support of the respective assignments. The observed conversion of 12α-deoxytetracycline into 4a,12a-anhydrodedimethylaminotetracycline by oxidative elimination of the dimethylamino group would *per se* favor the *cis* assignment.[10] Unfortunately, the low yield of this reaction together with the known tendency of the 4-dimethylamino group to undergo epimerization[11] may serve to minimize the stereochemical significance of the transformation.



Thus with elimination of N,N-dimethylhydroxylamine and the formation of another double bond, ring A would be stabilized by aromatization. The 4a,12a-anhydrodedimethylaminotetracycline (V) can be dehydrated easily in the presence of mineral acid. This affords the known compound, dedimethylaminoterrarubein (VI), which is reported as a degradation product from oxytetracycline,[4] and also has been prepared from the unrelated series of reactions cited in the accompanying publication.[7a]

Experimental[12]

12a-Deoxytetracycline (II).—Two grams of tetracycline hydrochloride (I) and 4 g. of zinc dust were combined in a pressure bottle with 50 cc. of 15% aqueous ammonia, and the mixture was agitated for 2 hours at room temperature. The excess zinc was filtered and the filtrate was very slowly neutralized with concentrated hydrochloric acid, while keeping the temperature below 20° by ice-bath cooling. At *p*H 7 the solids were removed by filtration and dissolved by acidification to *p*H 1.8 in 450 cc. water. The *p*H was adjusted to 4.2 with sodium hydroxide, and the insolubles at this point were separated on a Celite bed. The clear filtrate was extracted for 19 hours in a liquid-liquid extractor using ether as the solvent. The product which crystallized from the ether during extraction and upon concentration yielded 750 mg., yield 42%, m.p. 231–233° dec.

Anal. Calcd. for $C_{22}H_{24}N_2O_7$: C, 61.7; H, 5.6; N, 6.54. Found: C, 61.88; H, 5.92; N, 6.68.

The product was recrystallized by dissolving 250 mg. in 10 cc. of dimethylformamide, and diluting to 60 cc. with methanol. The product which crystallized weighed 230 mg. after drying at 60°.

Anhydro-12a-deoxydedimethylaminotetracycline.—A solution of 250 mg. of 12a-deoxytetracycline in 20 cc. of glacial acetic acid was diluted with 6 cc. of water and the system was alternately evacuated and flushed several times with nitrogen. Two grams of zinc dust was added slowly in small portions over a period of 3 hours. The reaction continued overnight after which the excess zinc was removed by filtration. An aliquot of the filtrate was shown to have an ultraviolet absorption spectrum identical with that of 12a-deoxydedimethylaminotetracycline. The reaction filtrate was diluted with five volumes of water and the solution was extracted with chloroform until the aqueous layer was colorless. The chloroform was back washed twice with water, dried over anhydrous sodium sulfate, and the solvent was evaporated to dryness in vacuum without heat. The solids thus obtained were dissolved in 4 cc. of glacial acetic acid. A few drops of 31% HBr in acetic acid was added which gave an immediate color change from yellow to orange. The anhydro-12a-deoxydedimethylaminotetracycline crystallized and after cooling was filtered, washed with ether and air-dried; wt. 140 mg. The product was dissolved in 4 cc. of hot dimethylformamide and the solution, after filtering, was diluted with an equal volume of hot methanol. The product crystallized out immediately, and was filtered after cooling, washed with methanol and dried at 60° *in vacuo;* wt. 61.4 mg. This compound was identical by all criteria with that described in the preceding paper.[7a]

(8) A. C. Cope, R. A. Pike and C. F. Spencer, THIS JOURNAL, **75**, 3212 (1953).

(9) C. W. Waller, B. L. Hutchings, R. W. Broschard, A. A. Goldman, W. J. Stein, C. F. Wolf and J. H. Williams, *ibid.*, **74**, 4981 (1952).

(10) Since this work was completed, a manuscript by S. Hitokawa, Y. Okaya, F. M. Lovell, and R. Pepinsky has come to our attention, which describes the X-ray crystallographic study of chlorotetracycline. This report presents convincing evidence for the *cis* relationship of the groups in question.

(11) (a) C. R. Stephens, L. H. Conover, P. N. Gordon, F. C. Pennington, R. L. Wagner, K. J. Brunings and F. J. Pilgrim, THIS JOURNAL, **78**, 1515 (1956); (b) J. R. D. McCormick, S. M. Fox, L. L. Smith, B. A. Butler, J. Reichenthal, V. R. Origoni, W. H. Muller, R. Winterbottom and A. P. Doerschuk, *ibid.*, **79**, 2849 (1957).

(12) We are indebted to Mr. L. Brancone and staff for analytical data, and to Mr. W. Fulmor and staff for spectral data.

4a,12a-Anhydrodedimethylaminotetracycline (V).—12a-Deoxytetracycline (50 mg.) was dissolved in 20 cc. of dimethoxyethane and 1.25 cc. of a chloroform solution of perbenzoic acid (12.9 mg./cc.) was added. The stoppered reaction solution was left to stand overnight at room temperature. The solvent was evaporated to about 1 cc. and the brown-red crystals which formed were filtered, washed with ether and dried at 60° *in vacuo;* wt. 8.48 mg., m.p. dec. slowly above 250°. This compound was identical by all criteria with that described in the preceding paper.[7a]

Anhydro-12a-deoxytetracycline Hydrobromide (IV).—Eight grams of 12a-deoxytetracycline was dissolved in 350 cc. of glacial acetic acid at 70° and the solution was treated with charcoal and filtered. To the filtrate, reheated to 70° was added 16 cc. of 31% HBr in glacial acetic acid. The color changed from yellow-orange to red, and within a few seconds the product deposited as light yellow flocculent crystals. The mixture was heated to 80° during which time the crystals changed to a more dense red colored solid. After heating for 15 minutes the mixture was cooled to room temperature, and the product was filtered, washed with acetic acid, chloroform, ether and dried at 60° in vacuum; wt. 7.46 g., m.p. 252° dec. The ultraviolet absorption spectra show $\lambda_{max}^{0.1 N\ HCl}$ 270 mμ, log ε 4.542; 425 mμ, log ε 3.922; $\lambda_{max}^{0.1 M\ Na_2B_4O_7}$ 268 mμ, log ε 4.572; 425 mμ, log ε 4.062.

Anal. Calcd. for $C_{22}H_{23}N_2O_8Br$: C, 53.8; H, 4.73; N, 5.72; Br, 16.32. Found: C, 53.15; H, 5.49; N, 5.29; Br, 17.22.

[CONTRIBUTION FROM THE DEPARTMENTS OF MICROBIOLOGY AND NEUROLOGY, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY, AND THE NEUROLOGICAL INSTITUTE, PRESBYTERIAN HOSPITAL, NEW YORK]

Immunochemical Studies on Blood Groups.[1] XXV. The Action of Coffee Bean α-Galactosidase on Blood Group B and BP1 Substances

BY MARIE LUISE ZARNITZ[2] AND ELVIN A. KABAT

RECEIVED JANUARY 29, 1960

Coffee bean α-galactosidase splits galactose from the blood group B and BP1 substances. The precipitating power of the B substance for human anti-B was reduced but its capacity to inhibit hemagglutination of B cells by anti-B was completely destroyed as was the precipitability of BP1 for human anti-BP1. Cross reactivity of both enzyme-treated substances with Type XIV horse antipneumococcal sera was increased. The implications of these findings for the structure of the B and BP1 substances are discussed.

Studies in several laboratories have established that the specificity of the blood group A, B and O(H) substances is associated with various oligosaccharide side chains which are split off by mild acid hydrolysis.[3-6] In the case of the blood group B substance, the specific side chains have a terminal, non-reducing galactose linked α- to the next residue. The evidence for this rests primarily on the observation that oligosaccharides and other galactosides having a terminal non-reducing galactose were more effective in inhibiting B-anti-B precipitation or the hemagglutination of B cells by anti-B than was galactose and that β-linked galactosides were less effective. No inhibition was observed with the other sugars present in the blood group substances.[7] Morgan[8] recently has reported that galactosyl-1,3-α-galactose was a better inhibitor of B-anti-B hemagglutination than was melibiose; galactose was not active under the conditions used. In addition, the action of an enzyme from *Trichomonas foetus* in destroying the activity of soluble blood group B substance was inhibited[9] by galactose, by methyl-α and β-D-galactopyranosides and by lactose and melibiose but not by other sugars and glycosides. Treatment of B substance with *T. foetus* enzyme split off galactose with the development of O(H) specificity[10a] and Iseki[10b] has obtained an enzyme from a strain named *Clostridium maebashi* which splits galactose from blood group B substance.

Another kind of terminal α-galactose may be exposed by mild acid hydrolysis of blood group B substance.[11] This procedure has been shown to liberate fucose and various oligosaccharides and, depending upon conditions, to destroy blood group specificity partially or completely. The non-dialyzable residue (BP1) from a B substance treated in this manner gave rise to antibodies on injection into a human of blood group A_1B. Precipitation of the BP1 substance by its antibody showed a specificity for α-galactosyl residues. Evidence that the α-galactosyl residues involved in B and BP1 specificity were linked differently to the second sugar unit was obtained.[11] The independence of the B, the BP1 grouping and the terminal β-linked galactoses responsible[12-14] for cross reactivity with Type XIV antipneumococcal sera could also be shown by time–hydrolysis curves and assaying for all three activities.[14]

Additional information about the B and BP1 reactive groupings could be obtained, were it possible to split off the terminal α-galactosyl residues and ascertain the effect on the various immunochemical properties. An α-galactosidase from coffee beans was described by Helferich and Vorsatz[15]

(1) Aided by grants from the National Science Foundation (G. 5208) and the William J. Matheson Commission.

(2) Rockefeller Foundation Fellow 1958–1959.

(3) E. A. Kabat, "Blood Group Substances," Academic Press, Inc., New York, N. Y., 1956; *Behringwerke-Mitteilungen*, **34**, 39 (1957).

(4) C. Howe, G. Schiffman, A. E. Bezer and E. A. Kabat, THIS JOURNAL, **80**, 6656 (1958).

(5) W. T. J. Morgan and W. M. Watkins, *Brit. Med. Bull.*, **15**, 109 (1959).

(6) W. T. J. Morgan, *Naturwissenschaften*, **46**, 181 (1959).

(7) E. A. Kabat and S. Leskowitz, THIS JOURNAL, **77**, 5159 (1955).

(8) W. T. J. Morgan, Conference on Mucopolysaccharides, Polish Academy of Sciences, Warsaw, October 1 to 3, 1959.

(9) W. M. Watkins and W. T. J. Morgan, *Nature*, **175**, 676 (1955).

(10) (a) W. M. Watkins, *Biochem. J.*, **64**, 21P (1956); (b) S. Iseki, personal communication to E. A. K., September 2, 1959.

(11) P. Z. Allen and E. A. Kabat, *J. Immunol.*, **82**, 340 (1959).

(12) M. Heidelberger, THIS JOURNAL, **77**, 4308 (1955).

(13) W. M. Watkins and W. T. J. Morgan, *Nature*, **178**, 1289 (1956).

(14) P. Z. Allen and E. A. Kabat, *J. Immunol.*, **82**, 358 (1959).

(15) B. Helferich and E. Vorsatz, *Z. physiol. Chem.*, **237**, 254 (1935).