The calculated molar ratios of these keto-acids are: KGA:PA:DMPA = 0.25:1.2:2.8.

The accumulation of these keto-acids was not due to acidification of the media, because they also occurred in biotin-deficient culture with calcium carbonate added. On the contrary, no accumulation of keto-acids occurred in biotin-rich media, or when preformed biotin-rich mycelial felts were floated on media adjusted to *p*H 4.6 with phosphoric acid. In thiamine-deficient culture, the accumulation of PA and KGA was observed but no DMPA could be found. The accumulation of DMPA is, therefore, to be attributed to biotin-deficiency.

Grateful acknowledgment is made to Prof. S. Tanaka for his interest and encouragement.

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REVERSIBLE ISOMERIZATIONS IN THE TETRACYCLINE FAMILY

Sir:

We have recently observed a reversible isomerization reaction creating for each of four members of the tetracycline family—chlorotetracycline,[1] bromotetracycline, oxytetracycline,[2] and tetracycline[3]—a new, isomeric substance. For each of the four tetracycline family members above, sets of conditions have been found catalyzing the formation of an equilibrium mixture of two components. For example, in the case of tetracycline itself, a twenty-four hour aging at 25° of a 15% tetracycline solution in 1 molar NaH_2PO_4 in 2:1 methanol–water (*p*H about 4.6) produced an equilibrium mixture judged spectrophotometrically and microbiologically to be about a 1.5:1 mixture of tetracycline and its new isomer, designated quatrimycin. Quatrimycin was isolated from the equilibrium mixture as the crystalline, homogeneous ammonium salt. Quatrimycin differs greatly in some of its properties from the starting tetracycline. For example, its isoelectric form is more water soluble. Its *in vitro* antibiotic activity against a variety of tetracycline-susceptible microörganisms is substantially less than that of tetracycline; for example, toward the turbidimetric assay using *E. coli*, quatrimycin shows 2–5% the activity of tetracycline. The possibility exists that the *in vitro* activity is actually zero, with partial equilibration under the test conditions accounting for the observed bioactivity. Re-equilibration of the isolated quatrimycin under the conditions used for tetracycline resulted in a reappearance of *in vitro* antibiotic activity and alteration in the ultraviolet absorption spectrum until the approximately 1.5:1 equilibrium mixture was again attained, from which both tetracycline and quatrimycin were re-isolated. The ultra violet absorption spectra, in 0.1 *N* HCl, for tetracycline and quatrimycin are presented in the graph.

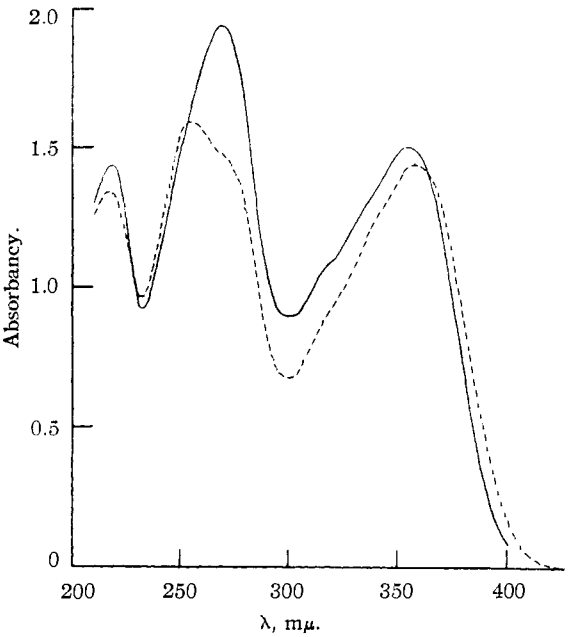

Fig. 1.—Tetracycline hydrochloride, 50.0 mg./l. in 0.1 *N* H_2SO_4——; quatrimycin, ammonium salt, 50.6 mg./l. in 0.1 N. H_2SO_4- - -.

Similarly, the equilibration of either tetracycline or quatrimycin can be accomplished in various buffers, such as formate, acetate or citrate, or in distilled water if sufficient time is allowed. The equilibrium can also be attained in various organic solvent systems, such as methanol. The solid crystalline materials are stable.

A similar series of observations holds for chlorotetracycline, bromotetracycline, and oxytetracycline. Their new isomers, chloroquatrimycin, bromoquatrimycin, and oxyquatrimycin, are all of lowered *in vitro* antibiotic activities and are changed in their ultraviolet spectra in the manner described for tetracycline. Preliminary animal work[4] shows each of the four new isomers to possess broad *in vivo* antibiotic activity.

(1) The trademark of the American Cyanamid Company for chlorotetracycline is Aureomycin.

(2) The trademark of Charles Pfizer and Company for oxytetracycline is Terramycin.

(3) The trademark of the American Cyanamid Company for tetracycline is Achromycin. The trademark of Charles Pfizer and Company for tetracycline is Tetracyn.

(4) This work was done under the direction of Dr. J. S. Kiser, Research Division, American Cyanamid Company.

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RECEIVED JULY 27, 1955

ON THE STEREOCHEMISTRY OF RESERPINE

Sir:

In a recent communication[1] we presented evidence for the relative and absolute configuration of four asymmetric centers (15, 16, 18 and 20) in reserpine (I).

(1) P. A. Diassi, F. L. Weisenborn, C. M. Dylion and O. Wintersteiner, THIS JOURNAL, **77**, 2028 (1955).