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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RECEIVED JULY 20, 1955

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.

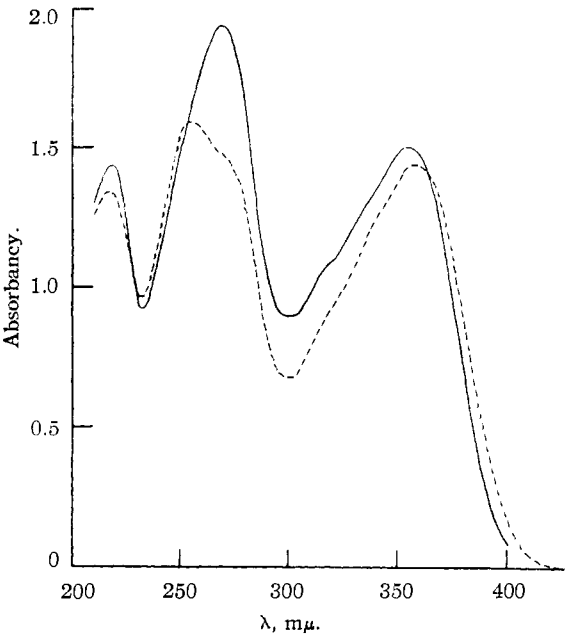

Fig. 1.—Tetracycline hydrochloride, 50.0 mg./l. in 0.1 *N* H_2SO_4——; quatrimycin, ammonium salt, 50.6 mg./l. in 0.1 N. H_2SO_4- - -.

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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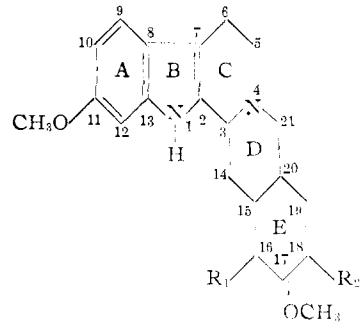
RECEIVED JULY 27, 1955

ON THE STEREOCHEMISTRY OF RESERPINE

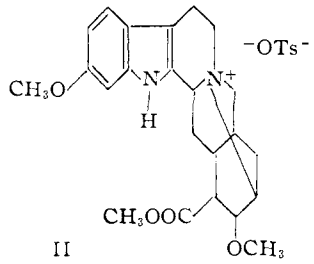
Sir:

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).

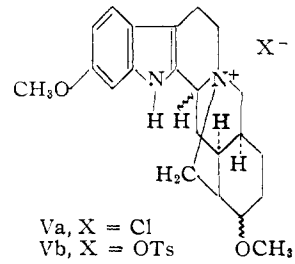
(1) P. A. Diassi, F. L. Weisenborn, C. M. Dylion and O. Wintersteiner, THIS JOURNAL, **77**, 2028 (1955).



I, $R_1 = -CO_2CH_3$, $R_2 = -O_2CC_6H_2(OCH_3)_3$
III, $R_1 = -CO_2CH_3$, $R_2 = -OSO_2C_7H_7$
IV, $R_1 = -CH_2OH$, $R_2 = -H$



II



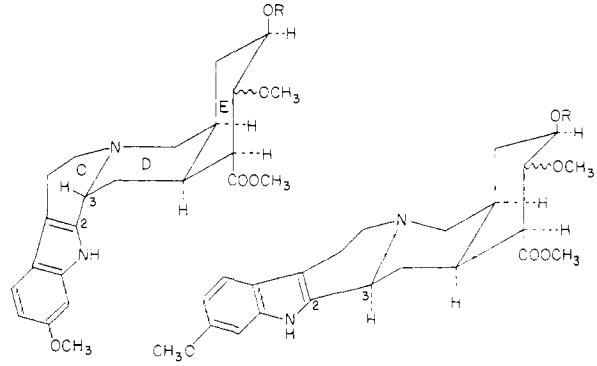
Va, X = Cl
Vb, X = OTs

The formation of the quaternary tosylate II from methyl reserpate 18-tosylate (III) on refluxing in collidine furnished conclusive proof that rings D and E in I are *cis*-linked. On the assumption that the displacement of the 18-tosyloxy group involves a concerted mechanism leading to inversion at C_{18}, we advanced the postulate that the 18-hydroxyl function and hence also the 16-carbomethoxy group[2] in I and III were *cis*-oriented in respect to the hydrogen atoms at the ring junction carbon atoms C_{15} and C_{20}. Application of Hudson's rotation rule to reserpine lactone[2a] then led to the assignment of the β-configuration to the functional group at C_{18} and hence also to the other substituents mentioned above.

In order to test the validity of the above assumption, we have recently essayed the preparation of the O-tosylate of the primary alcohol reserpinol (IV),[2b] since it was clear that the formation in this case of a quaternary salt would be incompatible with the postulated *cis*-relationship of the 16- and 18-substituents and the ring junction hydrogens. Indeed, elimination of the tosyloxy group occurred already on treatment of IV with *p*-toluenesulfonyl chloride in pyridine at room temperature. The crystalline product which deposited in 50% yield from the reaction mixture (m.p. 352–353°, $[\alpha]^{23}D$ +73.6° (*c*, 0.713 in water) after recrystallization from methanol) proved to be a quaternary chloride $C_{22}H_{29}N_2O_2Cl$ (calcd. C, 67.94; H, 7.52; N, 7.20; Cl, 9.12; found: C, 67.92; H, 7.53; N, 7.17; Cl, 8.90) by the following criteria: a free base could not be extracted from alkaline solution by organic solvents; it could not be titrated with perchloric acid in acetic acid solution, nor with base in aqueous solution as could be reserpinol hydrochloride (pK'_a 7.70); its infrared spectrum lacked a band in the 3.8 to 4.0 μ region characteristic for $>NH^+$.

From the pyridine mother liquor there was obtained after dilution with water and alkalinization a small amount of a chloroform-extractable substance which melted at 310–312° after recrystallization from methanol. The analysis (calcd. for $C_{29}H_{36}N_2O_5S$: C, 66.38; H, 6.92; N, 5.34; S, 6.11; found: C, 65.91; H, 6.51; N, 5.35; S, 5.59) and the infrared data (bands at 8.56, 8.95, 9.71 and 9.94 μ characteristic for the tosylate ion, no absorption in 3.8–4.0 region) suggest the quaternary tosylate corresponding to the above chloride.

It follows from these results that the hydroxymethylene group in IV and, hence, contrary to our previous postulate, the 16-carbomethoxy and 18-hydroxy functions in reserpine are *trans* to the C_{15} and C_{20} hydrogens (*i.e.*, the formation of the quaternary tosylate II from III occurred *with retention* of the configuration at C_{18}). Taking into account our previous deduction regarding the absolute configuration of this carbon atom,[1] the two quaternary salts obtained from reserpinol then have to be formulated as Va and Vb, respectively, with the ring junction hydrogen atoms α-oriented. Considering further that C_3 in reserpine is readily epimerizable,[3] and that its carbomethoxy group can be saponified with alkali without change of configuration[2a] and hence must be equatorial, we suggest that the stereochemistry of reserpine is best expressed by VI (C_2-C_3-axial to ring D) and that of its 3-epimer isoreserpine[3] by VII (C_2-C_3 equatorial to ring D).



VI VII

(2) (a) L. Dorfman, A. Furlenmeier, C. F. Huebner, R. Lucas, H. B. MacPhillamy, J. M. Mueller, E. Schlittler, R. Schwyzer and A. F. St. Andre, *Helv. Chim. Acta*, **37**, 59 (1954); (b) C. F. Huebner, H. B. MacPhillamy, A. F. St. André and E. Schlittler, THIS JOURNAL, **77**, 472 (1955).

(3) H. B. MacPhillamy, L. Dorfman, C. F. Huebner, E. Schlittler and A. F. St. André, *ibid.*, **77**, 1071 (1955).

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RECEIVED JULY 13, 1955

A COMPOUND WHOSE MOLECULE IS SUPERPOSABLE ON ITS MIRROR IMAGE BUT CONTAINS NO PLANE OR CENTER OF SYMMETRY

Sir:

The presence in any molecule of a plane or center of symmetry is a sufficient condition for optical inactivity,[1] and so far as we are aware, one

(1) We are here concerned only with optical activity in the fluid state.