Because plasmin is a more impure preparation than the chymotrypsin, the possibility was considered that inert proteins associated with the plasmin might protect the deoxyribonuclease from proteolytic attack. However, an equivalent amount of chymotrypsin added to the DNase-P mixture again completely destroyed the deoxyribonuclease in 1 hour. According to Feinstein and Hagen (6), ovalbumin had a remarkable effect in stabilizing deoxyribonuclease activity in crude pancreatic extracts; therefore, one per cent ovalbumin was added to a chymotrypsin-deoxyribonuclease mixture corresponding to the DNase-P formulation. The deoxyribonuclease was completely destroyed in 1 hour, indicating that the situation must be much different with the purified enzymes since this is even a higher level of inert protein than found in the DNase-P solution (about 0.6%). In this connection it might be noted that it is claimed in a recent patent (7) that solutions of crystalline deoxyribonuclease lost all activity in 3 hours at 31°. The deoxyribonuclease used in the present study showed no loss of potency in 4 hours at 37° in the presence or absence of plasmin, so that either the preparations or conditions used are not comparable in this respect. It is also now obvious that the possibility of chymotrypsin contamination must be ruled out in studies of the stability of any pancreatic deoxyribonuclease preparation.

By comparing the activities of bovine plasmin and chymotrypsin under optimum conditions of the fibrinolytic and caseinolytic assays, it was concluded that chymotrypsin "preferred" casein over fibrin as a substrate by a factor of about 3.5. In view of the above results it is apparent that the disparity may be orders of magnitude greater with respect to deoxyribonuclease (or perhaps some peptide bond related to its active center) as a substrate.

Under the conditions used for these stability studies plasmin was fairly unstable, presumably as a result of autodigestion in the absence of substrate. At the DNase-P level about 50% of the fibrinolytic activity remained after 1 hour and about 30% after 2 hours; in the case of chymotrypsin there was no significant decrease in this time. From the product processing point of view, therefore, it was possible to compensate for plasmin autodigestion by addition of excess plasmin without endangering the deoxyribonuclease activity; on the other hand, the amount of excess deoxyribonuclease required to compensate for its unexpectedly greater destruction by chymotrypsin eliminated consideration of such a mixture. The possibility of destruction of tissue deoxyribonuclease at the point of application by chymotrypsin and other proteolytic enzymes used in debriding preparations may have clinical implications, and it is hoped that information bearing on this question may be obtained in the future.

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Variation of pKa'-Values of Tetracyclines in Dimethylformamide-Water Solvents

By EDWARD R. GARRETT

The variation of pKa'-values for several tetracycline antibiotics with per cent dimethylformamide (DMF) in per cent DMF-per cent H_2O solvents (v/v) is given. Quantitative expressions for the pKa' dependence on per cent DMF for the various functional groups have been obtained. This information is utilized in the assignment of pKa'-values to functional groups.

THE DISSOCIATION CONSTANTS or pKa'-values of many of the tetracycline antibiotics have been determined in various nonaqueous-water solvents because of the difficulties of solubilization and maintenance of homogeneous solutions over the entire titratable range for one solvent alone (1–4). A frequently used solvent mixture has been dimethylformamide-water.

In addition, a traditional routine procedure has been to titrate a substance in a nonaqueous-water solvent with an aqueous titrant giving an apparent pKa' at half-neutralization difficult to compare with the results from other routine titrations, since amounts of titer may vary and the relation of pKa' with per cent nonaqueous solvent has not been established.

This communication presents information on the variation of pKa'-values for several tetracycline antibiotics, presents quantitative expressions for their dependence on per cent dimethylformamide (DMF) in %DMF–%H_2O solvents, and attempts to demonstrate their potential usefulness in deducing pKa' assignments to functional groups.

EXPERIMENTAL

The titrations of tetracycline free base and benzenesulfonyltetracyclinonitrile were conducted on 200 mg. samples in 20–50 ml. of solvents of varying %DMF–%H_2O composition at 25° with

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This study was done at the Research Division, The Upjohn Co., Kalamazoo, Mich.

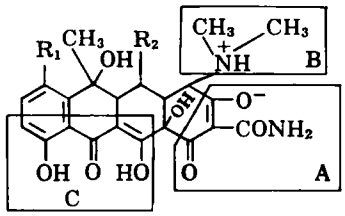
0.1000*N* HCl and 0.1*N* NaOH solutions. The titrations of desdimethylaminotetracycline and anhydrodesdimethylaminotetracycline plus added standard HCl were conducted on 10-mg. samples of the tetracyclines in 5 ml. %DMF–%H_2O solutions at 25° with 0.1000 *N* NaOH in a microburet.

The electrodes were glass-saturated calomel. The tetracycline derivatives were prepared by established methods (1–4) by Dr. Brian Bannister of the Upjohn Co.

The pKa'-values were estimated from the apparent pH values at half-neutralization of a functional group except for values *ca.* pKa' 4 and 10 where the Parke-Davis method was used (5, 6). The per cent DMF (v/v) at each pKa' was calculated from the known volume, the per cent of DMF (v/v) in the original solution and the known volume of aqueous titer added to achieve the apparent pH corresponding to that pKa'.

RESULTS AND DISCUSSION

The variation of pKa' for the various functional groups of tetracycline *Ic* as a function of the %DMF in %DMF–%H_2O (v/v) solvents is plotted in Fig. 1.



Ia, Oxytetracycline (Terramycin)
R_1 = H, R_2 = OH
Ib, Chlortetracycline (Aureomycin)
R_1 = Cl, R_2 = H
Ic, Tetracycline
R_1 = H, R_2 = H

The pKa'-assignments to the various labeled functional groups, A,B,C, of the tetracyclines *Ia*, *Ib*, *Ic* (1–4) are also designated by the lettered

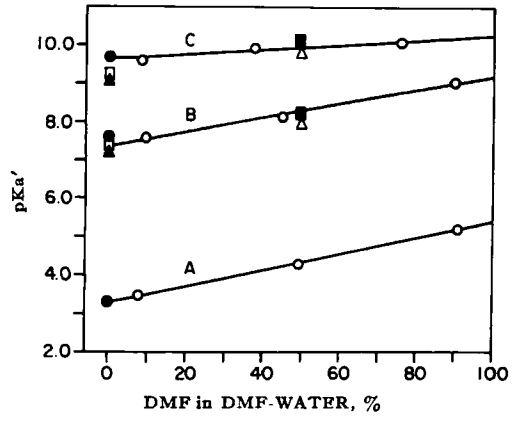


Fig. 1.—The pKa' of tetracycline *Ic* as a function of per cent dimethylformamide by volume in DMF–H_2O solvents. The open circles (○) are for the experimental data of this paper. The compounds, symbols, and references for other tetracyclines are: tetracycline, ● (4), ■ (3); oxytetracycline, △ (2), ▲ (4); and chlortetracycline, □ (4).

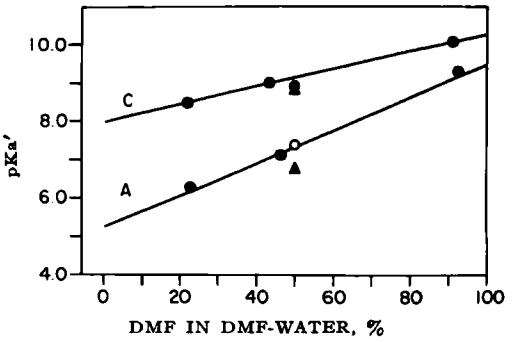


Fig. 2.—The pKa' of desdimethylaminotetracycline II as a function of per cent dimethylformamide by volume in DMF–H_2O solvents. The closed circles (●) are for the experimental data of this paper. The compounds, symbols, and references for other desdimethylaminotetracyclines are: desdimethylaminooxytetracycline, ▲ (2); and desdimethylaminochlortetracycline, ○ (3).

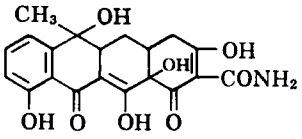
curves of Fig. 1.[1] Linear expressions[2] can be constructed to relate pKa' and %DMF for tetracycline *Ic* as

$$\text{Function A:}\quad pKa' = 0.021\,(\%DMF) + 3.30 \qquad \text{(Eq. 1a)}$$

$$\text{Function B:}\quad pKa' = 0.018\,(\%DMF) + 7.41 \qquad \text{(Eq. 1b)}$$

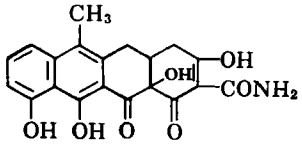
$$\text{Function C:}\quad pKa' = 0.007\,(\%DMF) + 9.62 \qquad \text{(Eq. 1c)}$$

The pKa'-assignments to the labeled functional groups of desdimethylaminotetracycline (hydrogen substituted for the dimethylamino group in *Ic*) are designated by the lettered curves of Fig. 2 (1-4).



II

Desdimethylaminotetracycline



III

Anhydrodesdimethylaminotetracycline

The linear expressions constructed to relate pKa' and %DMF for desdimethylaminotetracycline II are

[1] The assignments used are as published in literature (1–4). However, in light of recent interest in possible reassignment of pKa' values, it is interesting to note that the classical increase of apparent pKa' with decreasing dielectric constant for uncharged acids could argue for the interchange of the assignments of functions B and C. (See Fig. 1.)

[2] Due to a possible change in selective solvation of the dissociating molecule as 0% DMF is approached, the intercept of such expressions may vary slightly from the observed pKa' values in water.

Function A: $pKa' = 0.042\ (\%DMF) + 5.25$ (Eq. 2a)

Function C: $pKa' = 0.023\ (\%DMF) + 8.00$ (Eq. 2c)

Some of the literature data on pKa' (1–4) for the various tetracyclines in %DMF–$\%H_2O$ solutions are also plotted in Figs. 1 and 2 for comparison. It is apparent that the aureomycin and terramycin tetracyclines have lower pKa'-values but a similar relation with %DMF so that Eqs. 1 and 2 may be used as satisfactory estimates of pKa'-variation with %DMF—the same slopes but slightly lower intercepts.

A pKa' of 7.35 in 80%DMF–20%H_2O was observed for 10-benzenesulfonyltetracyclinonitrile. (The nitrile is substituted for the carboxamido group in *Ic*.) This value is consistent with the previously reported pKa' 6.9 at 50%DMF (2,4) for 10-benzenesulfonylterramycinonitrile which was assigned to the dimethylammonium ion. Addition of HCl to 10-benzenesulfonyltetracyclinonitrile showed no titratable group below pH 5 and above pH 2.0. This was consistent with the premise of highly increased acidity of the zwitterionic enolic system with nitril derivatives similar to but more acid than A in I(4).

The anhydrodesdimethylaminotetracycline, III, was observed to have two pKa'-values, pKa_1 = 8.06 (94%DMF), pKa_2 = 10.26 (91%DMF). This compound is analogous to anhydrodesdimethylaminoterramycin previously reported (2).

Now the pKa'-values of anhydroterramycin in water can be given (2) as 3.8 for A, 5.5 for C, and 7.2 for B, where the analogous functionalities are given in I. Since a 1,8-dihydroxybenzophthalide system has a pKa of 4.7 (2), the 5.5 pKa can possibly be assigned to the similar group C in anhydroterramycin; the 7.2 pKa' is consistent with the dimethylamine grouping B, zwitterionic with the carboxamide grouping A.

Removal of the dimethylamino group may consistently decrease the acidity of the carboxamido-enol complex A. It is noted that the pKa' 4.3 is elevated to 7.4, Δ pKa' = 3.1 in 50%DMF, and the pKa' 5.3 is elevated to 9.3, Δ pKa' = 4.0 in 95%DMF, for the change from tetracycline to desdimethylaminotetracycline (Figs. 1 and 2). Using these Δ pKa'-values, it can be predicted that on the insertion of a dimethylamino group into anhydrodesdimethylamino tetracycline III, the now zwitterionic carboxamide-enol complex could be assigned either the pKa'-values of 10.3 − 4.0 = 6.3, or 8.1 − 4.0 = 4.1 at 95%DMF. Because the derived pKa' of 6.3 for 95%DMF is the more consistent of these two possible values with the pKa' of the carboxamido-enol complex of tetracycline in 95%DMF, *i.e.*, 5.4 implies that the pKa_2 = 10.3 (94%DMF) is better assigned to the carboxamido grouping A in III, and the pKa_1 = 8.1 (94%DMF) can be assigned to the dihydroxynapthalene grouping C in III.

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Effect of Zinc Oxide Catalyzed Peroxide on Sterols of Almond, Cottonseed, and Olive Oils

By M. WAFIK GOUDAH and EARL P. GUTH

The phytosterols of almond, cottonseed, and olive oils undergo changes in structure when the oils are irradiated in both ultraviolet and sunlight with zinc oxide. The changes in the phytosterol molecules of the oils indicate a probability that a similar change could occur in the sterols of the skin. These changes might produce a molecule with anti-inflammation activity, thus providing an explanation for the dermatological effect of zinc oxide external preparations.

THE CATALYTIC ACTIVITY of zinc oxide in the photochemical formation of hydrogen peroxide has been confirmed by many workers (1, 2). The pharmaceutical aspects of the phenomenon have also been investigated by Guth, Reese, Mathias, Minardi, Blubaugh, Young, and Lozada (3–8).

Lozada and Guth (8) showed that when zinc oxide-containing ointments were irradiated with ultraviolet light in the presence of water and air the measurable peroxide was less or nonexistant in systems that contained cholesterol or other sterols. This was particulary true when wool fat or hydrophilic petrolatum were components of the ointment base. This lack of peroxide was accounted for by the effect on the sterol molecule.

Infrared spectrographic studies showed that the β-hydroxyl group on cholesterol was oxidized to a carbonyl group. There was also evidence that the double bond shifted from the 5:6 position to the 4:5 position.

With this as a background, a study of the effect of zinc oxide catalyzed peroxide on the sterols in

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