

Studies in the Tropolone Series

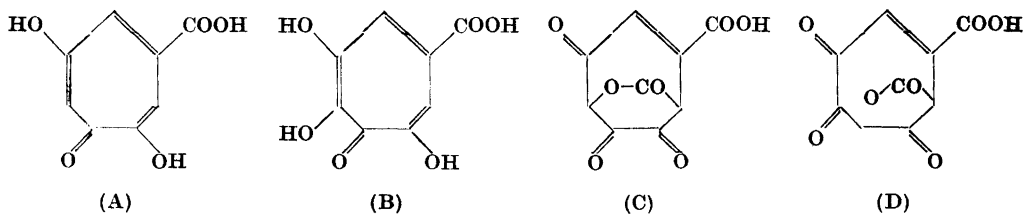
IV. Stipitatic, Puberulic and Puberulonic acids

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In a previous communication¹ ultra-violet absorption spectra were reported for α -, β - and γ -thujaplicins in neutral and alkaline solutions, and some general physico-chemical properties of the tropolone ring system were discussed in the light of this and other data. As briefly mentioned in a recent note², the investigation has now been extended to the natural compounds stipitatic, puberulic and puberulonic acids.

These acids were first isolated from mould cultures by Raistrick *et al.*^{3, 4}, and as a result of their studies these workers concluded that stipitatic acid might "belong to a class of compounds not previously encountered among mould metabolic products, except possibly for puberulic acid"³. Stipitatic and puberulic acids have recently been shown by Todd and co-workers to have the structures (A)^{5, 6} and (B)^{5, 7} respectively (or tautomerides thereof) in conformity with the earlier suggestions of Dewar^{8, 9}.

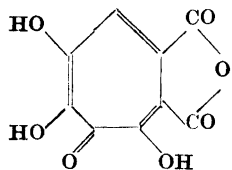


Birkinshaw and Raistrick⁴ ascribed the formula $C_8H_4O_6$ to the yellow puberulonic acid, which they believed to be intimately related to puberulic acid. However, they did not succeed in transforming one compound into the other. Todd and co-workers showed^{5, 10, 7} that puberulonic acid, on heating

to 100° in aqueous solution, loses one molecular equivalent of CO₂ and is converted into puberulic acid. The formula was accordingly amended to C₉H₄O₇.

Todd *et al.*^{5,7} proposed structure (C) or an isomeric γ -lactone structure (D) for puberulonic acid. These formulae were based on certain chemical properties of the substance, *e. g.* its easy conversion into puberulic acid, its reported resistance towards acetylating agents⁴, and the formation of a condensation product with *o*-phenylenediamine^{5,10}.

The light absorption properties of puberulonic acid, however, favour another structure (E) for the substance, or a tautomeric form, still containing the tropolone ring. This structure was originally proposed² on the basis of the ultra-violet investigation to be reported in this paper, and it has now been confirmed by infra-red spectrographic studies¹¹.



(E)*

A preliminary comparison of the U. V. absorption in organic solvents of stipitatic, puberulic and puberulonic acids and the thujaplicins showed a remarkable similarity between them all. This, together

with Dewar's previous suggestion of tropolone structures for stipitatic⁸ and puberulic⁹ acids, suggested to the present author that puberulonic acid also might be a tropolone derivative (*cf.* Raistrick, Bakerian lecture, May 1949¹²).

Ultra-violet absorption curves for aqueous solutions of stipitatic⁶, puberulic¹⁰ and puberulonic¹⁰ acids have been published by Todd *et al.* and similar results (Fig. 1) were obtained in these laboratories except for puberulic acid**, for which a sharper short-wave maximum was found. The general similarity in light absorption is obvious from these curves, but it is evident that water of undefined pH is not a suitable solvent for a detailed investigation of the absorption properties of easily ionizable substances.

The structures (C) and (D) contain α -diketone groupings, which will cause a yellow colour. Low absorption maxima in the visible region are exhibited by α -diketones in general (*e. g.* diacetyl in hexane: $\log \epsilon = 1.3$ at ca 430 m μ ¹³; camphorquinone in *cyclohexane*: $\log \epsilon = 1.6$ at 478 m μ ¹⁴). Puberulonic acid, however, shows a much more intense long-wave absorption (Figs. 1 and 8), which cannot be explained by the simple —CO—CO— chromophore of (C). Likewise in (D), the unsaturated system —CO—CO—CH = C(COOH)— is branched and not necessarily planar and thus constitutes a complex chromophore that could hardly give rise to the intense long-wave absorption of puberulonic acid.

* The symbol (E) will subsequently be used to indicate any of the four tautomerides.

** According to a private communication from Professor Todd (January 18th, 1950), the published extinction curve for puberulic acid in water is erroneous. He does not intend to publish a correction, and accepts the revised data discussed herein.

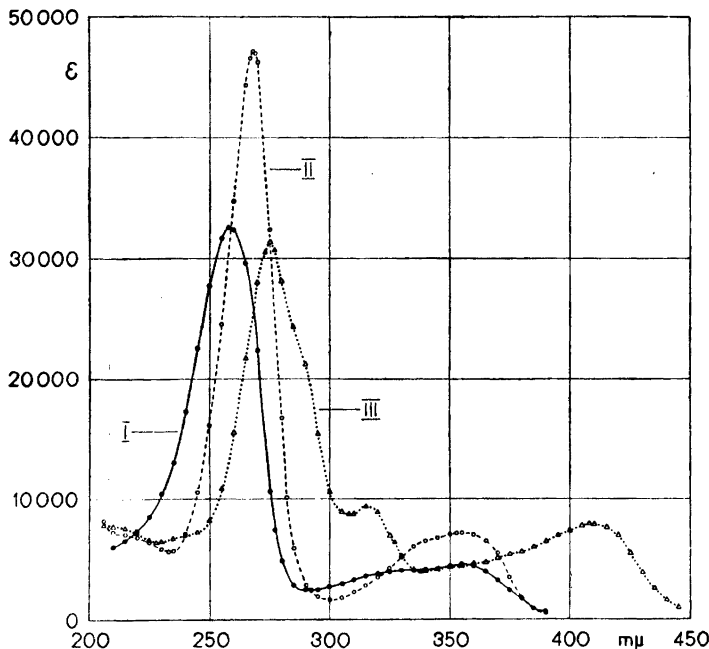


Fig. 1. *Stipitatic acid (I), puberulic acid (II), and puberulonic acid (III) in aqueous solution.*

Considerations of this kind necessitated the construction of a new formula as a working hypothesis. (E) is the only structure that combines the composition $C_9H_4O_7$ with the tropolone skeleton and can explain the transformation into puberulic acid. Attempts were made to confirm this structure by a study of the hydrolysis and dissociation of puberulonic acid, and particularly the ultra-violet absorption data for the various ions. For comparative purposes and in view of the chemical as well as the spectral¹ relationship between aromatic substances and tropolones, it was also of interest to study the absorption properties of stipitatic and puberulic acids, following step by step the influence of ionization of the various acidic groups by using buffered solutions of adequately varied pH.

EXPERIMENTAL

Prior to investigation, the stipitatic acid specimen * was recrystallized three times from water. This had no significant effect upon the absorption. The puberulic and puberulonic acid samples were used without further purification.

* Pure samples of the three acids were kindly provided by Professor H. Raistrick.

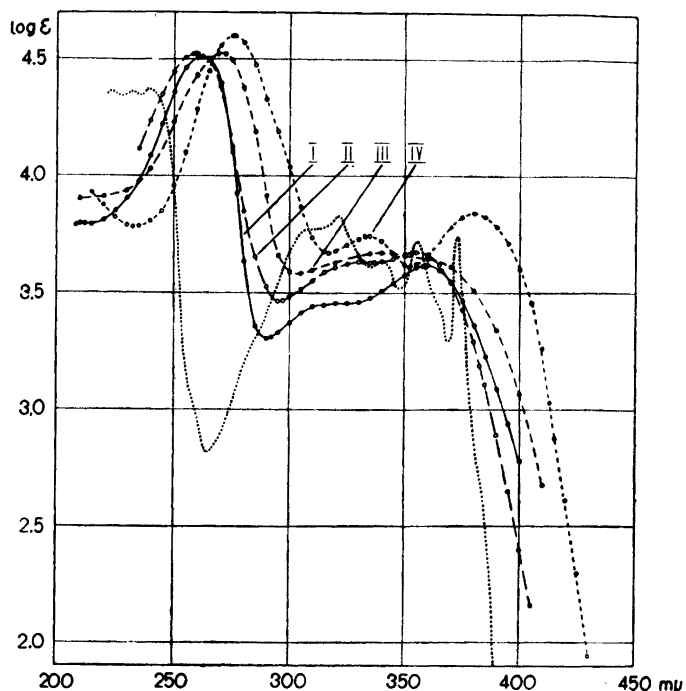


Fig. 2. *Stipitatic acid in N-hydrochloric acid (I), at pH 4.8 (II), at pH 7.9 (III), and at pH 12.0 (IV). Dotted line: tropolone in cyclohexane¹.*

The following solvents were employed: distilled water, absolute ethanol, purified for spectral purposes¹⁵, dioxan, refluxed with potassium hydroxide and fractionated, phosphate buffer solutions of pH 5.7, 7.7, 7.9, 11.2 and 12.0, phosphate — citric acid buffer solutions of pH 3.5, 4.4 and 4.8, and an ammonia — ammonium chloride buffer solution of pH 8.2.

A Beckman Model DU spectrophotometer with calibrated cells was used for the measurements. The absorption properties of some of the alkaline solutions gradually changed on storing, so that, unless otherwise stated, these solutions were investigated as soon as possible after preparation — at critical wavelengths within a few minutes.

RESULTS AND DISCUSSION

Stipitatic, puberulic and puberulonic acids are all almost insoluble in saturated hydrocarbons. A direct comparison of the undissociated compounds with tropolone and the thujaplicins in such solvents is therefore impossible.

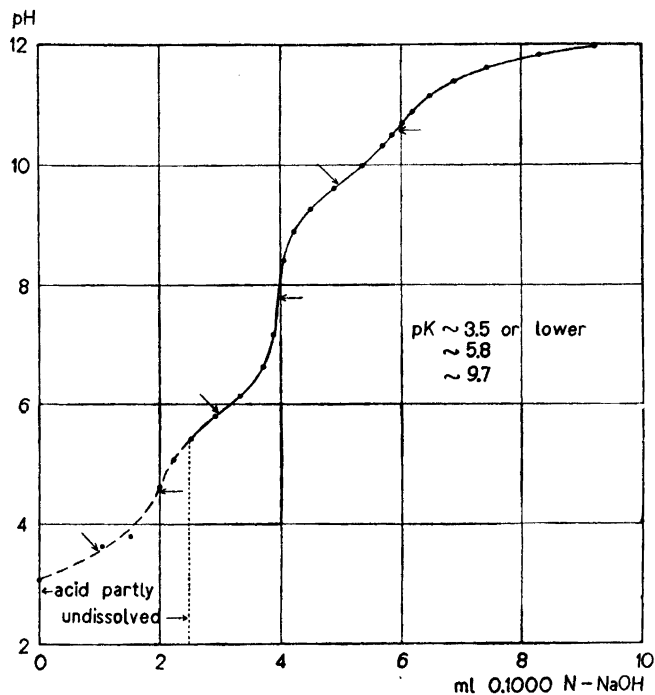


Fig. 3. Potentiometric titration of stipitatic acid.

The extinction curve of unionized stipitatic acid in *N*-hydrochloric acid solution shows the general features of the curve for tropolone in *cyclohexane*¹ (Fig. 2), except for vibrational fine structure. However, the absorption intensity at about 300–325 $m\mu$ is considerably diminished, and the short-wave absorption band exhibits a combined hyperchromic and bathochromic displacement, obscuring the deep minimum of the tropolone curve. It is seen that the simultaneous introduction of a hydroxyl and a carboxyl group into tropolone — in contrast to benzene¹⁶ — does not cause a bathochromic displacement of the long-wave absorption band.

The effect of dissociation of the various acidic groups of stipitatic acid was studied on the basis of the potentiometric titration curve (Fig. 3), obtained by adding sodium hydroxide solution to a well-stirred aqueous suspension of the acid. In Fig. 3, horizontal arrows indicate equivalence points: the lower ones giving at the same time the pH values for maximum concentrations of the monovalent and bivalent ions. Curves II and III in Fig. 2 are the corresponding extinction curves, whereas IV is the curve for fully ionized stipitatic acid.

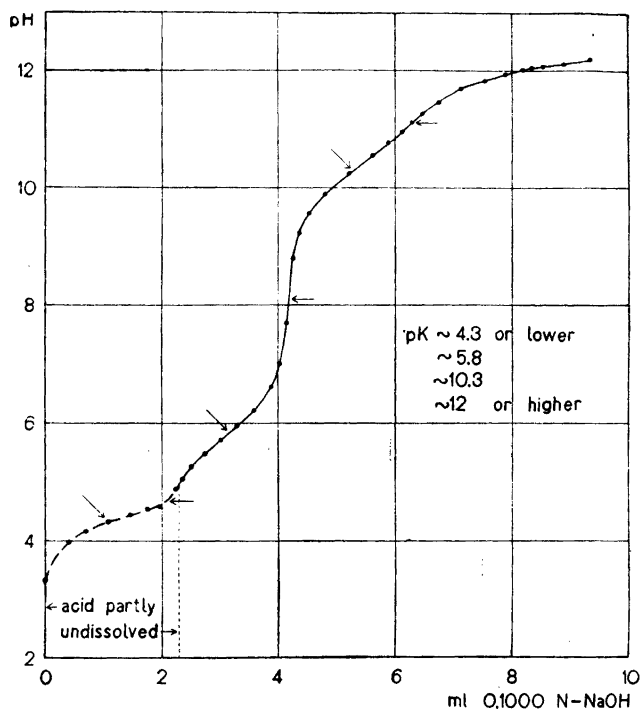


Fig. 4. Potentiometric titration of puberulic acid.

There can be no doubt that the lowest pK value must be assigned to the dissociation of the carboxyl group. The isolated hydroxyl group must be assumed to come next in acidity — pK ca 5.8 — whereas the other hydroxyl group (in m -position to the carboxyl or to the isolated hydroxyl group, depending upon the tautomeride preferred) will be more weakly acidic due to the influence of the neighbouring oxygen atom. The pK value of about 9.7 found for this hydroxyl group also conforms with the expectation that dissociation of the “olone” hydroxyl group must take place with greater ease in tropolone itself (pK ca 7¹⁷⁻¹⁸) than in a negative ion of stipitatic acid.

Comparison of the curves I and II shows that the dissociation of the carboxyl group causes a small hypsochromic shift, as is usual with carboxylic acids. However, dissociation of the first hydroxyl group (curve III) reverses this effect, displacing the entire curve about 15 $m\mu$ towards the red. The last dissociation step (curve IV), involving the “olone” hydroxyl group, leads to a further bathochromic displacement, splitting of the long-wave band into two separate peaks, and a considerable increase in ϵ , especially in the band close

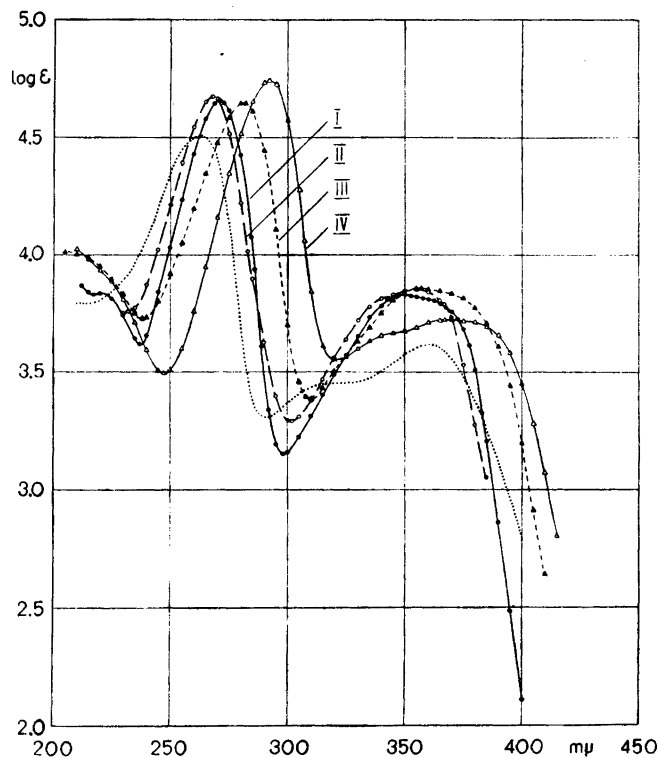


Fig. 5. Puberulic acid in *N*-hydrochloric acid (I), at pH 4.4 (II), at pH 8.2 (III), and at pH 11.2 (IV). Dotted line: stipitatic acid in *N*-hydrochloric acid.

to the visible region. The division of the broad long-wave absorption band into two peaks recalls the effect of ionization upon the extinction curves of the thujaplicins¹, and is probably characteristic of the elimination of the proton from the "olone" grouping of the tropolones.

Puberulic acid, being a hydroxy-stipitatic acid, contains four ionizable groups. Its titration curve, obtained in the same way as the one for stipitatic acid, is shown in Fig. 4. Like formula (A) for stipitatic acid, formula (B) for puberulic acid is only one of the two possible tautomerides, both containing the tropolone skeleton, which can be constructed for this acid. Under these circumstances it is not possible to assign with certainty each of the pK values of 5.8 and higher to an individual hydroxyl group. It can only be stated that the dissociation having pK 5.8 refers to a hydroxyl group non-adjacent to the CO-group.

Fig. 5 shows the extinction curve for unionized puberulic acid and the approximate curves for its mono-, di-, and tri-valent anions. Puberulic acid has a

steeper extinction curve than stipitatic acid (Fig. 5), the short-wave maximum is displaced about 10 $m\mu$ towards the red, and there is no indication of a maximum at about 315 $m\mu$. Tropolone and the thujaplicins¹ have prominent maxima in this region, and in stipitatic acid the maximum still exists although it is less intense. Its disappearance in the case of puberulic acid may perhaps be due to the influence of the neighbouring hydroxyl group on the „olone” system.

Dissociation of the carboxyl group of puberulic acid causes the well-known slight hypsochromic displacement (Fig. 5, curve II). The effect of dissociation of the first hydroxyl group is also the expected one — a marked red-shift of the entire curve. The next ionization step (curve IV, pH 11.2) produces not only another shift in the same direction but also an intensification of the short-wave maximum and an indication of a division of the long-wave band into two peaks.

It has not been possible to demonstrate the effect of dissociation of the last hydroxyl group of puberulic acid, since in strongly alkaline media as for example N-sodium hydroxide solution the substance is too unstable to allow a determination of the absorption curve by ordinary methods. The extinction curve obtained by a rapid determination at pH 12.0 was, however, very similar to that found at pH 11.2.

Puberulonic acid is not unaffected by alkali. Its solutions have been reported to undergo various colour changes on alkalisation and reacidification^{19,10}. Barger and Dorrer¹⁹ attempted to determine the basicity but obtained ambiguous results. Recently, Todd *et al.*¹⁰ have published a potentiometric titration curve for the substance showing that it undergoes some change on storing at any pH between approximately 7 and 10. It was suggested that this change might involve the opening of a lactone ring.

The potentiometric titration of puberulonic acid has been repeated in these laboratories, using about 20 mg of the acid in 20 ml of water. The titration extended over several days altogether and gave a result differing from that mentioned above. In Fig. 6, filled dots indicate the pH readings for the solution at equilibrium; open dots indicate the initial pH values after each addition of alkali. The acid dissolved gradually when the first mole of sodium hydroxide was added, and the pH readings plotted in Fig. 6 were only reached after some minutes of stirring. At pH 3.6—3.7 all the acid was dissolved. During the addition of the second mole of alkali (pH 4—6), the initial pH values were still not stable, indicating that some change in the molecule was gradually taking place. The end values were reached within about ten minutes in the beginning of this interval, in about an hour at the end. At about pH 6 the solution had become almost colourless. Addition of the third mole of alkali

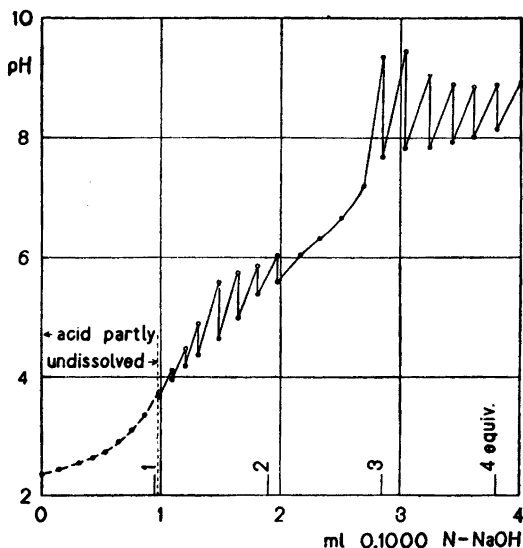
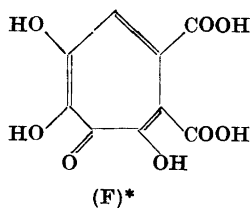


Fig. 6. Potentiometric titration of puberulonic acid.

gave no abnormal effect. The fourth mole caused another reaction, which was, however, much slower than the first one, and since oxygen was not excluded, may have involved an oxidation. The nature of this process was not studied further.

The reaction taking place at pH 4—6 can be explained on the basis of the structure (E) as a gradual hydrolysis of the anhydride to give the corresponding dibasic acid (F), or the tautomeric forms, a process which is complete at pH 5.6. The titration cannot show whether hydrolysis takes place to any considerable extent even below pH 4, because, if it occurred in this region it would be a rapid process and its pH effect would be obscured by that of the dissolution of the substance.



In order to get a clear picture of the process of hydrolysis we must consider the equilibrium between the anhydride and the diacid in aqueous solution as well as the ionization of both (E) and (F). At a low pH practically no ions are present but only uncharged molecules (E) and (F) in unknown proportions. As the pH increases, the relative proportions of these molecules remain, of course, unchanged, whereas their total concentrations diminish due to ion formation. After the addition of one mole of alkali most of the material is present in the form of the monovalent ions "E⁻" and

* (F) hereafter symbolizes any one of the four tautomerides.

"F⁻", the proportion of each depending upon the unknown values of the equilibrium and dissociation constants concerned. The reactions that take place on further addition of alkali must involve the transformation "E⁻" → "F⁻" (via intermediates), since the second ionization constant of (F) must be higher than that of (E). At pH 5.6, the solution at equilibrium will contain predominantly the ion "F⁻", which is then ionized further on addition of more alkali.

In order to verify this interpretation of the titration data for puberulonic acid, the U. V. absorption curves were determined for solutions of this substance in an inert solvent (dioxan) and in a series of buffered aqueous solutions, the pH values of which were selected with reference to the titration curve. The extinction curves obtained for solutions at equilibrium are shown in Fig. 7.

Curve I in Fig. 7 (dioxan solution) must be that of the anhydride with no appreciable admixture of the dibasic acid. In Fig. 8 it is reproduced, together with the curves for tropolone, stipitatic and puberulic acids in non-ionizing solvents. A comparison shows that the main difference between the curve for puberulonic acid and that for puberulic acid is a marked red-shift of the whole curve. This conforms with the presence of an extra substituent in the nucleus and the conjugation of the anhydride ring (*cf.* below).

The second absorption curve in Fig. 7 (pH 0) is exhibited, not only by solutions obtained by dissolving puberulonic acid in N-hydrochloric acid directly, but also when a freshly prepared solution of pH 14 is acidified to pH 0. The absorption of the anhydride at approximately 295 and 315 m μ is still distinguishable in this strongly acidic aqueous solution. However, two new maxima appear at 277 and 375 m μ . These are located at shorter wavelengths than the corresponding anhydride maxima just mentioned. This is in agreement with the assumption that they are caused by the dicarboxylic acid (F), since other anhydride — diacid pairs, *e. g.* maleic²⁰ and phthalic²¹, show similar absorption differences. A comparison of curve II with the dotted absorption curve of undissociated puberulic acid (Fig. 7) also supports this interpretation.

The distribution of absorption intensity along curve II indicates the remarkable situation that the anhydride and the acid are present in comparable concentrations. Although organic acid anhydrides are in general rapidly and completely transformed into the corresponding acids on treatment with water, some cases of slow hydrolysis are well known, as for example phthalic anhydride. An interesting sequence of increasing stability towards water was described by Rivett and Sidgwick²²: maleic anhydride is hydrolyzed rapidly, citraconic anhydride more slowly, and pyrocinchonic anhydride in aqueous solution gives an equilibrium with only a small proportion of pyrocinchonic

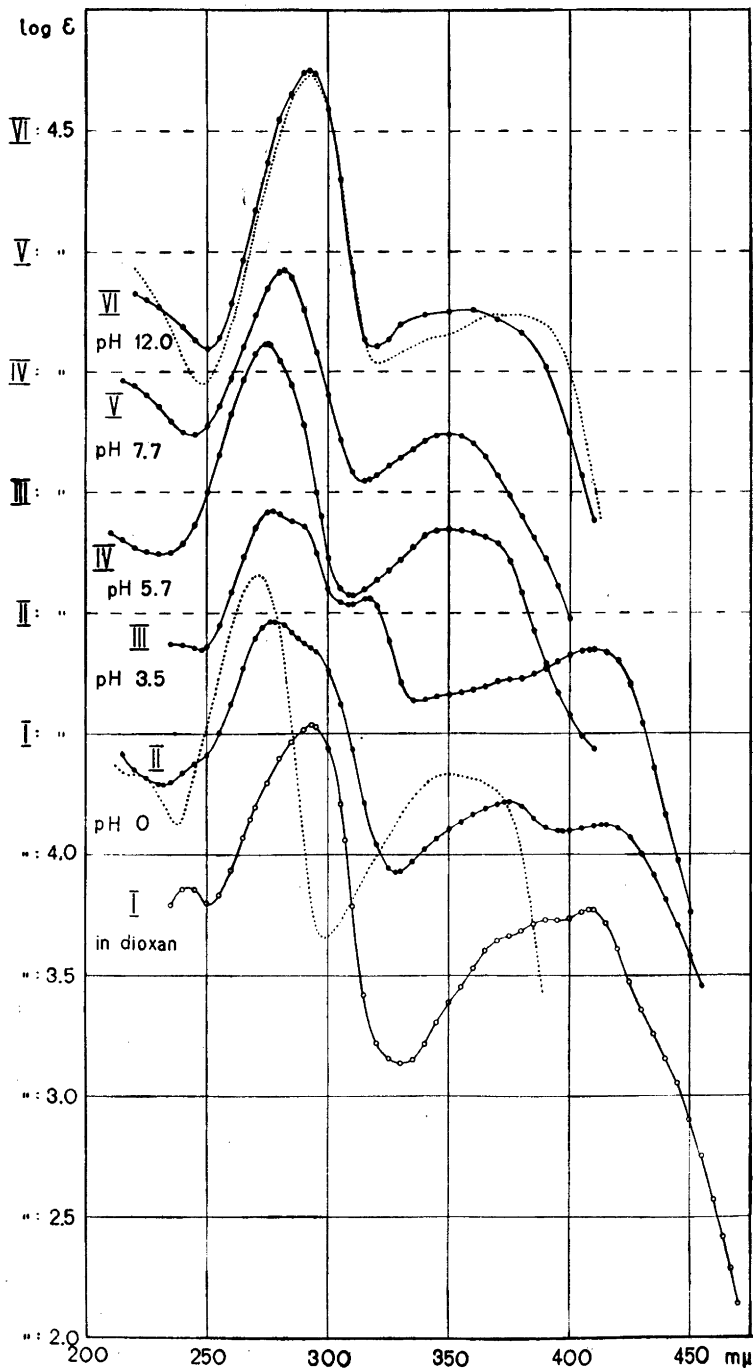


Fig. 7. Puberulonic acid in dioxan (I), in *N*-hydrochloric acid (II), at pH 3.5 (III), at pH 5.7 (IV), at pH 7.7 (V), and at pH 12.0 (VI). Dotted lines: puberulonic acid in *N*-hydrochloric acid and at pH 12.0 respectively.

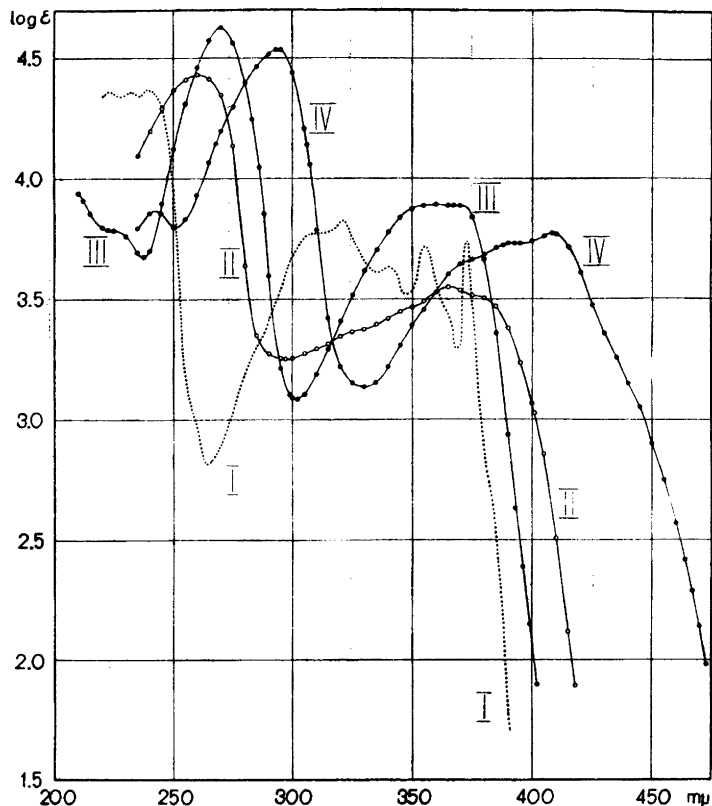


Fig. 8. Tropolone in cyclohexane¹ (I), stipitatic acid in dioxan (II), puberulic acid in ethanol (III), and puberulonic acid in dioxan (IV).

acid. Verkade²³ reported that tetramethyl succinic anhydride passes away with the vapour on boiling a solution of tetramethyl succinic acid, and Δ^1 -tetrahydrophthalic acid²⁴ exhibits the same phenomenon. Other examples of anhydrides that are stable in the presence of water are some highly substituted fulgides²⁵ and cantharidin²⁶.

In none of these cases have the proportions of acid and anhydride in equilibrium at room temperature been reported to be approximately equal. The proportion of anhydride seems to increase with temperature.

A common feature of the anhydrides mentioned above is substitution of the hydrogen atoms of the anhydride ring by larger groups, which probably increase the stability of this ring by steric or other influences. The seven-membered ring of puberulonic acid may well exhibit the same effect.

The third absorption curve in Fig. 7 is that of the equilibrium mixture at pH 3.5. According to the above interpretation of the titration curve this mixture should contain mainly the ions "E⁻" and "F⁻" together with some of the uncharged molecules (E) and (F) and the ion "F⁻". The new and striking feature of curve III is the maximum at 317 m μ , which is not present in any of the other curves, but is found in ordinary aqueous solutions of puberulonic acid (Todd *et al.*¹⁰ and Fig. 1). Very probably it is due to the monovalent anion of the anhydride (E), in spite of the fact that it lies further from the strongest maximum of undissociated puberulonic acid (curve I) than would be expected on the basis of the demonstrated effect of hydroxyl ionization of stipitatic and puberulic acids (Figs. 2 and 5). A support of this interpretation is provided by the fact that the maximum at 317 m μ is more intense immediately after the addition of excess of buffer solution of pH 3.5 to a dioxan solution of the substance. Moreover, the maximum is absent immediately after acidifying an alkaline solution to pH 3.5. The explanation of these phenomena is, of course, that ionization takes place instantaneously, whereas the equilibrium (E) \rightleftharpoons (F) is established only after some time.

The maximum at 277 m μ in curve III together with the flat absorption at about 350 m μ must be due mainly to the ion "F⁻". The "shoulder" at 290 m μ is probably caused by some undissociated anhydride present. Ionization of (E) apparently causes some increase of absorption in the visible region.

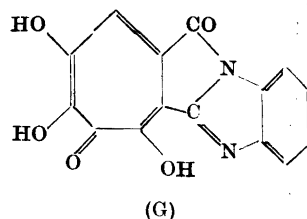
Curve IV (Fig. 7) corresponds to the point on the titration curve where two moles of alkali have been added and the anhydride has been completely transformed into the dibasic acid. The doubly charged anion "F⁻" should, therefore, predominate in this solution. This conforms very well with the extinction curve, which shows no indication of the presence of the anhydride or its anion. The short-wave maximum has undergone the slight hypsochromic shift expected to occur upon dissociation of the carboxyl groups.

In the solution of pH 7.7 (curve V) the first dissociation of a hydroxyl group in the acid has taken place, resulting in a red-shift of the short-wave maximum. Further dissociation (curve VI) increases this effect and brings the curve very close to that of the trivalent anion of puberulic acid (dotted line).

Thus, it is seen that the ultra-violet absorption data are entirely consistent with the tropolone anhydride structure for puberulonic acid. The lactone formulae, on the other hand, do account for the formation of the acid (F) in alkaline solution, but hardly explain the establishment of an equilibrium in aqueous solution between approximately equal parts of puberulonic acid and (F). An equilibrium involving one of the lactones is improbable from the point of view of energy considerations, because the tropolone ring, being almost aromatic in nature, must possess a high resonance energy. Moreover, on the

basis of formula (C) or (D) a feasible interpretation cannot be found for the maximum at 317 $m\mu$.

Chemical evidence is not necessarily against the anhydride formula. The acetylating difficulties may be overcome by finding suitable conditions, and the condensation product with *o*-phenylenediamine^{5,10} is not necessarily a quinoxaline. The most probable structure is (G) — or a tautomeric form (*cf.* above) — which would be analogous to the products obtained by condensing *o*-phenylenediamine with, for example, cantharidin²⁷, diphenylmaleic anhydride²⁸ or hexahydro phthalic anhydride²⁹.



SUMMARY

Stipitatic, puberulic and puberulonic acids have been titrated potentiometrically and their U. V. absorption properties have been investigated in organic solvents, and in aqueous buffer solutions of various pH. The influence of the dissociation of the various acidic groups of stipitatic and puberulic acids on the U. V. absorption curves conforms on the whole with expectations based on their structures and previous work on related substances.

It has been shown that the lactone formulae suggested for puberulonic acid by other workers are not compatible with its properties as demonstrated in the present work. Instead, a tropolone anhydride formula (E) is proposed to account for its spectral similarity to stipitatic and puberulic acids, for the anomalies encountered in titration of the substance, and for its U. V. absorption properties at various hydrogen ion concentrations. Spectral evidence discloses the existence of an equilibrium in aqueous solution at room temperature between comparable amounts of puberulonic acid and the corresponding dicarboxylic acid.

REFERENCES

1. Aulin-Erdtman, G. *Acta Chem. Scand.* 4 (1950) 1031.
2. Aulin-Erdtman, G. *Acta Chem. Scand.* 4 (1950) 1325.
3. Birkinshaw, J. H., Chambers, A. R., and Raistrick, H. *Biochem. J.* 36 (1942) 242.
4. Birkinshaw, J. H., and Raistrick, H. *Biochem. J.* 26 (1932) 441.
5. Corbett, R. E., Hassall, C. H., Johnson, A. W., and Todd, A. R. *Chem. and Ind.* (1949) 626.
6. Corbett, R. E., Johnson, A. W., and Todd, A. R. *J. Chem. Soc.* (1950) 147.
7. Corbett, R. E., Johnson, A. W., and Todd, A. R. *J. Chem. Soc.* (1950) 6.
8. Dewar, M. J. S. *Nature* 155 (1945) 50.
9. Dewar, M. J. S. *Nature* 155 (1945) 479.

10. Corbett, R. E., Hassall, C. H., Johnson, A. W., and Todd, A. R. *J. Chem. Soc.* (1950) 1.
11. Aulin-Erdtman, G., and Theorell, H. *Acta Chem. Scand.* 4 (1950) 1490.
12. Raistrick, H. *Proc. Roy. Soc. A* 199 (1949) 141.
13. Lardy, G. C. *Compt. rend.* 176 (1923) 1548.
14. Lowry, T. M., and Simpson, D. M. *J. Chem. Soc.* (1936) 1156.
15. Weigert, F. *Optische Methoden der Chemie.* Leipzig (1927) 212.
16. Castille, A., and Klingstedt, F.-W. *Compt. rend.* 176 (1923) 749.
17. von E. Doering, W., and Knox, L. H. *J. Am. Chem. Soc.* 72 (1950) 2305.
18. Cook, J. W., Gibb, A. R., Raphael, R. A., and Somerville, A. R. *Chem. and Ind.* (1950) 427.
19. Barger, G., and Dorrer, O. *Biochem. J.* 28 (1934) 11.
20. Wassermann, A., and Smakula, A. *Z. phys. Chem. A* 155 (1931) 366.
21. Menczel, S. *Z. phys. Chem.* 125 (1927) 161.
22. Rivett, A. C. D., and Sidgwick, N. V. *J. Chem. Soc.* 97 (1910) 1677.
23. Verkade, P. E. *Rec. trav. chim. Pays-Bas* 40 (1921) 199.
24. Baeyer, A. *Ann.* 258 (1890) 145.
25. Stobbe, H. *Ber.* 38 (1905) 3673.
26. Homolka, B. *Ber.* 19 (1886) 1082.
27. Gadamer, J. *Arch. Pharm.* 260 (1922) 199.
28. Bistrzycki, A., and Fässler, K. *Helv. Chim. Acta* 6 (1923) 519.
29. Betrabet, M. V., and Chakravarti, G. C. *J. Indian Chem. Soc.* 7 (1930) 191.

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