Ultraviolet Absorption Spectra of Creatinine

ALBERT WOLLENBERGER*

*Institute of Biochemistry, University of
Uppsala, Sweden*

Grinbaum and Marchlewski¹ have reported that the ultraviolet absorption curve of creatinine in water exhibits a maximum at 234.5 $m\mu$ and a minimum at 228.7 $m\mu$, though the curve published by them shows the minimum to lie approximately 10 $m\mu$ lower. These authors have further reported that the maximum at 234.5 $m\mu$ is enhanced in acid and abolished in alkali. According to the data presented below the latter findings are both erroneous. Bandow's statement² that the ultraviolet absorption spectrum of creatinine in water shows little which is worth of interest is likewise misleading.

Fig. 1 shows the absorption of ultraviolet light by creatinine between 210 and 290 $m\mu$ at various ranges of pH. The absorption measurements were taken with a Hilger Uvispek photoelectric spectrophotometer. The creatinine was a Kebo, Stockholm, product and was recrystallized from alcohol. A concentration of 10^{-4} molar in buffer, HCl, KOH, or water was used, with the solvent serving as the blank. The temperature was 22° C. Curve 2 of the figure is the absorption curve of creatinine in water and in buffer solutions and dilute alkali of pH 6.5–12.3. The position and intensity of the absorption maximum ($\lambda = 234$ $m\mu$; $\epsilon = 6.9 \times 10^3$) agree well with the values given by Grinbaum and Marchlewski¹ for creatinine in "acid". The curve has a minimum at 216 $m\mu$. Addition of acid does not enhance the absorption at 234 $m\mu$, as was claimed by Grinbaum and Marchlewski, but dimi-

Fig. 1

nishes it and causes a shift of the maximum to lower wave lengths. The hypsochromic effect is not surprising and may be attributed to fixation of the distribution of the π -electrons by the positive charge conferred upon the molecule in acid. At pH 3 and below the ultraviolet absorption spectrum of creatinine is represented by curve 1, which has a maximum at 217 $m\mu$ ($\epsilon = 4.5 \times 10^3$). An increase in pH above 12.3 produces at first a shift of the maximum of curve 2 to lower wavelengths with a simultaneous increase of the maximum. In 0.9–1.5 *N* hydroxide curve 3 is obtained, the maximum of which ($\epsilon = 11.1 \times 10^3$) lies at 225 $m\mu$. On increasing the alkali concentration above 1.5 *N* the absorption peak is shifted back to higher wave lengths. Thus in 10 *N* KOH it lies at 229 $m\mu$ and in 16 *N* KOH at 231.5 $m\mu$. Creatine, which under these conditions is generated from creatinine at appreciable rates, exhibits no comparable absorption in this ultraviolet region.

* Fellow of the Life Insurance Medical Research Fund, New York, U.S.A.

Absorption curve 1 is obviously that of the creatinium ion and curve 2 that of the neutral molecule, while curve 3 represents chiefly the ion with a single negative charge. The bathochromic shift on increasing the alkali concentration above 1.5 *N* corresponds probably to the acquisition of a second negative charge. The existence of equilibria between the different ionic and molecular species is demonstrated by the fact that the described changes in the ultraviolet absorption spectrum with change in pH are all reversible. From the spectrophotometric data the apparent constant of the basic ionization equilibrium of the neutral molecule was calculated, the pK'_a obtained being 5.02. The pK'_a of the acidic ionization is in the neighborhood of 13.4.

For analytical applications the absorption curve of the neutral creatinine has the advantage that it occurs over a wide range of pH and that its $\lambda_{\max}$ lies well within the limits of the recording instruments commonly in use. The absorption at this wave length was found to obey Beer's law over a concentration range of 8×10^{-6} — 2×10^{-4} *M*. Making use of this absorption we have determined creatinine and compounds convertible into creatinine (creatine and phosphocreatine) in biological material, following their separation from other chromogenic substances. This is being reported elsewhere. A more detailed account of the present work, which is also to include evidence concerning the specific chromophores responsible for the various absorption bands shown, will be presented at a later date.

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1. Grinbaum, R., and Marchlewski, L. *Bull. intern. acad. polon. Classe sci. math. nat. Sér. A.* **1937**, 156.
2. Bandow, F. *Biochem. Z.* **299** (1939) 199.

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Occurrence of Betulinic Acid in *Menyanthes trifoliata* L.

ARNULV STABURSVIK

*Institutt for Organisk Kjem, Norges Tekniske
Høgskole, Trondheim, Norway*

During the last years the triterpene betulinic acid, first found in nature by Robertson, Soliman and Owen (1939)¹, has been isolated from a series of plants, and various previously isolated substances have been shown to be identical with this acid. Betulinic acid may be very difficult to obtain in a crystalline state, and recrystallization of the crude acid does not always remove all impurities. Thus it is possible that this substance in many instances has been overlooked and may prove to be more common than is thought today.

It can be seen from Table 1 that betulinic acid has a rather scattered distribution throughout the plant kingdom, and does not seem to be characteristic of certain families. Remarkable is the report that, in *Alyxia*, betulinic acid is found to occur in specimen from low-rainfall areas away from the coast, whereas in coastal forms of the same plant it is reported to be replaced by ursolic acid and oleanolic acid².

Betulinic acid has been found in the leaves of *Alyxia* and in the leaves and the stems of *Nuytsia*², in the seeds of *Zizyphus*³, and in the rhizomes of *Menyanthes* (this paper). In the other plants listed in the table it has been isolated from the bark.

As first pointed out by Barton and Jones⁶, it seems highly probable that gratiolone, isolated from the drug *Herba Gratiola officinalis* (*Scrophulariaceae*) by Retzlaff⁷, is identical with betulinic acid. Different substances isolated from *Platanus* spp. (lit. quoted by⁴) are probably also betulinic acid, as may be true for a substance isolated from *Cornus sanguinea* L. by Zellner and Fajner⁸.