Absorption curve 1 is obviously that of the creatininium 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 reverrible. 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 $8 \times 10^{-6} - 2 \times 10^{-4}$ $M$. 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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# Occurrence of Betulinic Acid in *Menyanthes trifoliata* L.

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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 acid [2].

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 Jones [6], it seems highly probable that gratiolone, isolated from the drug *Herba Gratiola officinalis (Scrophulariaceae)* by Retzlaff [7], is identical with betulinic acid. Different substances isolated from *Platanus spp.* (lit. quoted by [4]) are probably also betulinic acid, as may be true for a substance isolated from *Cornus sanguinea* L. by Zellner and Fajner [8].

*Table 1.*

*Loranthaceae:*
*Nuytsia floribunda* (Labill.) R.Br. [2]
*Platanaceae:*
*Platanus acerifolia* Willd. [4]
*Myrtaceae:*
*Melaleuca rhaphiophylla* Schau. [2]
*M. cuticularis* Labill. [2]
*M. viminea* Lindl. [2]
*M. leucadendron* L. [2]
*M. parviflora* Lindl. [2]
*M. pubescens* Schau. [2]
*Syncarpia laurifolia* Tenn. [5]
*Rhamnaceae:*
*Zizyphus vulgaris* Lamark *var. spinosus* Bunge [3].
*Cornaceae:*
*Cornus florida* L. [1]
*Gentianaceae:*
*Menyanthes trifoliata* L.
*Apocyanaceae:*
*Alyxia buxifolia* R. Br. [2]

*Experimental.* (All melting points are corrected.) Fresh rhizomes of *Menyanthes trifoliata*, collected in shallow waters in a lake near Trondheim in October, 1949, were continuously extracted with hot ethanol in a Soxhlet extractor. A green gelatinous mass precipitated from the extract. It was filtered off and washed thoroughly, first with water and then, after drying, with petroleum ether (b.r. 60–90°), which removed most of the chlorophyll. The residue was recrystallized from methanol (charcoal) until colourless. Yield about 1 g per kg of fresh rhizome.

The isolated substance crystallised as fine needles, m.p. 304–7°; $[\alpha]_D^{20}$ + 10° (ethanol; *C*, 0.62; *l*, 2 dm). *M* (by titration) 459, 467. Calculated for $C_{30}H_{48}O_3$: *M* 457.

Acetylation, by the method of [1], gave the mixed anhydride of acetylbetulinic acid and acetic acid. The crude crystals melted at 193–94°. After 3 recrystallisations from diluted ethanol, acetylbetulinic acid, m.p. 293–94°, was obtained. Saponification gave betulinic acid, m.p. 313°.

For comparison acetylbetulinic acid was prepared from betulin by the method of Ruzicka, Lamberton and Christie [9]. M.p. 292–93°. Mixed m.p. 292.5–93.5°. The X-ray powder diagrams were identical.

The evaporated alcoholic extract from *Menyanthes* amounts to about 10 per cent of the fresh weight, and consists to a great extent of saponins. Whether these contain betulinic acid has not yet been determined.

I wish to express my thanks to Professor N. A. Sörensen for his continuous interest during this investigation, and to Dr. H. Sörum for preparing the powder diagrams. I am indebted to *Norges Tekniske Högskole* for a research fellowship.

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## Paper Strip Identification of Phenyl Thiohydantoins

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In Edman's method [1] for determining the amino acid sequence in peptides phenyl thiohydantoins are formed from the amino acids. For identification, the former are hydrolysed by hot alkali to the corresponding amino acids. However, certain amino acids are decomposed by this treatment, making the identification ambiguous or impossible. This applies to serine, threonine, cystine, arginine, asparagine, and glutamine. This difficulty could be avoided by a direct identification of the phenyl thiohydantoins. A procedure to this purpose using paper chromatography is reported here.