*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.

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1. Robertson, A., Soliman, G., and Owen, E. C. *J. Chem. Soc.* **1939**, 1267.
2. Anstee, J. R., Arthur, H. R., Beckwith, A. L., Dougall, D. K., Jefferies, P. R., Michael, M., Watkins, J. C., and White, D. E. *J. Chem. Soc.* **1952**, 4065.
3. Kawaguti, R., and Kim, K. W. *J. Pharm. Soc. Japan* **60** (1940) 343; *Chem. Abstr.* **35** (1941) 1396.
4. Bruckner, V., Kovàcs, J., and Koczka, I. *J. Chem. Soc.* **1948**, 948.
5. Ralph, C. S., and White, D. E. *J. Chem. Soc.* **1949**, 3433.
6. Barton, D. H. R., and Jones, E. R. H. *J. Chem. Soc.* **1944**, 659.
7. Retzlaff, F. *Arch. Pharm.* **240** (1902) 561; *Chem. Zentr.* **1903 : I**, 42.
8. Zellner, J., and Fajner, R. *Monatsh.* **46** (1925) 611; *Chem. Zentr.* **1926 : II**, 599.
9. Ruzicka, L., Lamberton, A. H., and Christie, E. W. *Helv. Chim. Acta* **21** (1938) 1706.

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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.

*Experimental.* The descending technique was used throughout. For localisation of the spots on the paper a modification [2] of the iodine-azide reaction described by Feigl [3] was used. This reaction is said to be specific for divalent sulphur. The test is highly sensitive and care should be taken to avoid contamination by extraneous sulphur (*e. g.* rubber).

*Pretreatment of paper.* Whatman No. 1 filter paper was used. It was found advantageous to impregnate the paper with the starch prior to use. Water soluble starch was freed from metal ions by extracting it first with 2.5 % ethanolic 8-hydroxyquinoline and then several times with ethanol. The filter paper was soaked in a 0.5 % aqueous solution of this starch preparation and then dried at 40—50° C for about one hour.

*Solvents.* A) 70 ml heptane (dist., b.p. 98° C) and 30 ml pyridine (dried over pellets of KOH and dist.). B) 40 ml heptane, 20 ml *n*-butanol (dried over CaO and dist.) and 40 ml 90 % formic acid (Baker's Analyzed) were shaken in a separatory funnel. The upper layer was used in the trough and the lower for saturating the atmosphere. C) 40 ml heptane, 40 ml *n*-butanol and 20 ml 90 % formic acid.

For a satisfactory and reproducible result it was necessary to equilibrate the paper with a saturated atmosphere of the solvent before starting the chromatography. This was particularly important with the solvents B and C. In order to reduce the equilibration time it is recommended to hang in the chromatographic chamber a piece of cloth soaked with the solvent.

*Localisation of the spots.* The paper was dried at 90—100° C for 5—10 minutes. The iodine-azide reagent was then applied in a finely divided spray. The reagent was made up immediately before use by mixing equal volumes of an iodine solution (*M*/100 $J_2$, *M*/2 KJ in water) and an azide solution (*M*/2 $NaN_3$ in water). The hydantoins revealed themselves as bleached areas on a dark blue background. As little as 0.5 μg could easily be detected.

The table shows the $R_F$-values of a number of phenyl thiohydantoins prepared from naturally occurring amino acids *. It can be seen that one—dimensional runs in these solvents permit the unambiguous identification of every hydantoin. The small difference in $R_F$-values between the leucine and isoleucine hydantoins was found to be consistent.

| Phenyl thiohydantoin of | $R_F$-value in solvent A | B | C |
|---|---|---|---|
| Arginine | 0 | 0 | 0.46 |
| Aspartic acid | 0.05 | 0.05 | 0.71 |
| Histidine | 0.05 | 0 | 0.41 |
| Asparagine | 0.06 | 0.02 | 0.57 |
| Glutamine | 0.06 | 0.02 | 0.61 |
| Glutamic acid | 0.10 | 0.06 | 0.75 |
| Lysine ** | 0.16 | 0.04 | 0.86 |
| Tyrosine | 0.19 | 0.15 | 0.81 |
| Tryptophan | 0.24 | 0.41 | 0.83 |
| Hydroxyproline | 0.24 | 0.14 | 0.73 |
| Glycine | 0.26 | 0.20 | 0.69 |
| Alanine | 0.37 | 0.40 | 0.80 |
| Phenylalanine | 0.40 | 0.63 | 0.87 |
| Methionine | 0.40 | 0.54 | 0.85 |
| Threonine | 0.47 | 0.63 | 0.87 |
| Proline | 0.48 | 0.58 | 0.87 |
| Valine | 0.51 | 0.67 | 0.88 |
| Isoleucine | 0.59 | 0.74 | 0.90 |
| Leucine | 0.61 | 0.75 | 0.91 |

* The phenyl thiohydantoins from serine and cystine are not included since pure specimens have not been available.

** ε-phenyl thioureido-.

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1. Edman, P. *Acta Chem. Scand.* **4** (1950) 283.
2. Chargaff, E., Levine, C., and Green, C. *J. Biol. Chem.* **175** (1948) 67.
3. Feigl, F. *Qualitative analysis by spot tests*, 3rd Ed., New York, 1947.

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