Our further effort was to quantitate these results, and these are summarized in the Table[10].

Zusammenfassung. Aus dem nativen löslichen und unlöslichen Kollagen wurden drei Ketosäuren, und zwar Brenztraubensäure, α-Ketoglutarsäure und α-Ketoisovaleriansäure isoliert. Diese Ketosäuren häufen sich in den peripheren Gebieten des Kollagenmoleküls (in Telopeptiden)[7,8] an.

J. Rosmus[11] and Z. Deyl[12]

Department of Biology, Massachusetts Institute of Technology, Cambridge (Massachusetts, USA),
16th February 1967.

---

[10] This research was supported by grant No. NB 00024 from the National Institute of Neurological Diseases and Blindness, Department of Health, Education and Welfare, U.S. Public Health Service and by a grant from The Medical Foundation of Boston.

[11] Present address: Central Research Institute of Food Industry, Prague-Smíchov (Czechoslovakia).

[12] Present address: Laboratory of Gerontology, Physiological Institute, Czechoslovak Academy of Sciences, Prague-Krč (Czechoslovakia).

Anthocyanins of *Dioscorea alata* L.

The greater yam, or water yam, *Dioscorea alata* L., is an important food crop in parts of South-East Asia, and has been introduced by cultivation to many other tropical countries, including West Africa. The flesh of the edible tubers of most cultivars is white, but some contain a purplish-red pigment. The whole tuber may be deeply coloured, or there may be only a thin s.c. coloured layer, the greater part of the tuber being faintly pink or white: the leaves and stems of the growing plant also are sometimes suffused with pigment. These types have been separated as distinct species, *D. atropurpurea* Roxb., *D. purpurea* Roxb. and *D. rubella* Roxb.[1], but are now generally regarded simply as varieties of *D. alata*[2]. The pigment is usually assumed to be an anthocyanin, but no information as to its composition appeared in the literature, and the authors are unaware of reports of anthocyanins in any of the Dioscoreaceae.

Tubers of a purple-fleshed form of *D. alata* grown on the University Farm, Legon, have been examined for the presence of anthocyanins. The pigments were extracted by homogenizing 20 g of peeled tuber in 100 ml of methanolic 1% HCl for 5 min. The suspension was left overnight, the extract filtered off and the whole process repeated. The combined extract was shaken with an excess of purified ether. The pigments were separated as an aqueous concentrate, washed successively with light petroleum and benzene, and then exhaustively extracted with ethyl acetate.

Further purification and separation of the anthocyanin pigments was undertaken by paper chromatography[3]. The purified extract was banded on several sheets of Whatman No. 3 paper and developed with 1:1 *n*-butanol-2*N* HCl. 3 distinct coloured bands, 1 being much stronger than the others, appeared.

The anthocyanin bands were cut out, eluted with 5% acetic acid in methanol and after concentration in vacuo at 30–35 °C, purified by re-running in *n*-butanol-acetic acid-water (62:12:26) and then in 15% aqueous acetic acid on washed paper. Aqueous solvent was used to remove free sugars[4].

The purified eluates were concentrated in vacuo and shaken with small amounts of 20% HCl in a large excess of ether. The pigment was transferred completely into the aqueous layer, which was separated, traces of ether and methanol removed in vacuo, and then heated on a boiling water bath for 3 min. The solution was quickly cooled, extracted with amyl alcohol, and the organic layer washed with water. Amyl alcoholic extracts of the aglycones were then applied to Whatman No. 1 paper. 4 solvent systems were used for development: (1) 'Forestal solvent' – water-acetic acid-conc. HCl (10:30:3 v/v); (2) Acetic acid-conc. HCl-water (5:1:5 v/v); (3) *m*-cresol-5,5*N* HCl-water (1:1:1 v/v); (4) *n*-butanol-2*N* HCl (1:1 v/v, top layer). The *n*-butanol: 2*N* HCl solvent mixture was kept, while the paper was equilibriated with the lower aqueous phase, for 24 h, before use.

On all the chromatograms of hydrolysed pigment extracts only a single anthocyanidin spot appeared; this spot was magenta in visible light and bright pink in UV-light. The Rf value of this anthocyanidin was compared with those of known anthocyanidins[4–8] (see Table I), and found to correspond with cyanidin. This was confirmed by co-running with a sample of cyanidin,

Table I. Rf values of the principal anthocyanidins and of *D. alata* anthocyanidin in different solvent systems

Anthocyanidin	Bu:HCl	'Forestal'	AcOH:HCl	*m*-cresol:HCl
Pelargonidin	0.80	0.68	0.55	0.82
Cyanidin	0.69	0.50	0.34	0.69
Peonidin	0.72	0.63	0.50	0.87
Delphinidin	0.35	0.30	0.22	0.52
Petunidin	0.45	0.45	–	0.75
Malvidin	0.53	0.60	0.43	0.90
Hirsutidin	0.72	–	–	–
Anthocyanidin of *D. alata*	0.69	0.50	0.35	0.70

---

[1] G. Watt, *A Dictionary of the Economic Products of India* (W. H. Allan and Co., London 1890).

[2] D. Prain and I. H. Burkill, Ann. R. bot. Gdn Calcutta *14*, 211 (1939).

[3] J. B. Harborne, Nature *181*, 26 (1958).

[4] J. B. Harborne, J. Chromat. *1*, 473 (1958).

[5] E. C. Bate-Smith and R. G. Westall, Biochim. biophys. Acta 4, 427 (1950).

[6] E. C. Bate-Smith, Biochem. J. *58*, 122 (1954).

[7] Abe Y. Hayashika and S. Mitsui, Proc. Japan Acad. *34*, 373 (1958).

[8] K. Hayashika, Pharmazie *12*, 245 (1957).

when no separation occurred. All 3 anthocyanins thus have the same aglycone and are cyanidin glycosides.

The sugar components were identified by a method used for the identification of the sugar components of cocoa anthocyanins[9]. Each methanolic eluate of the individual anthocyanins from Whatman No. 3 paper was evaporated to dryness in vacuo. The anthocyanins were hydrolysed for 30 min in $0.5 N$ sulphuric acid in a boiling water bath. The hydrolysates were neutralized with $BaCO_3$, centrifuged and concentrated in vacuo. The residue was extracted with methanol and the extract concentrated in vacuo to approximately 0.2 ml. The sugar chromatograms were developed in ethyl acetate-pyridin-water (2:2:1 v/v)[10], the sugar spots detected by spraying with aniline-phthalate reagent[11], and compared with standard sugars.

Table II. Rf values of the principal cyanidin glycosides and of *D. alata* anthocyanin in different solvent systems

Cyanidin glycoside	ACOH-HCl-W	ACOH-HCl-W	BAW	HCl-W
	5:1:5	82:15:3	4:1:5	3:97
3-glucoside	0.61	0.26	0.38	0.07
3-galactoside	0.61	0.26	0.37	0.07
3-rhamnoglucoside	–	0.43	0.37	0.19
3-gentiobioside	–	–	0.29	–
3-xyloglucoside	0.85	0.51	0.36	0.24
3-diglucoside	–	0.61	0.33	0.34
3,5-diglucoside	0.70	0.40	0.28	0.16
3-rhamnoglucosido-glucoside	–	0.59	0.25	0.36
Major anthocyanin of *D.* alata	0.69	0.38	0.28	0.14

The sugars obtained from the major anthocyanin and from one of the minor anthocyanins could not be separated from glucose. The concentration of the third was so low that it was not possible to determine the sugar component with the amount of sample available. On the chromatograms a trace of arabinose was detected but this may be an artefact formed during the chromatography[12].

The Rf values of the anthocyanins isolated were compared with those of known cyanidin glycosides (Table II). The major component of the pigment is thus shown to be cyanidin-3-5-diglucoside.

The Rf values of the 2 minor anthocyanins when run in non-alcoholic solvents were similar to the Rf values of cyanidin-3-monoglucoside and cyanidin-3-rhamnoglucoside, but when run in BAW the Rf values did not coincide. Further studies on the 2 minor yam pigments are therefore necessary.

Résumé. Trois anthocyanines ont été extraits de tubercules d'une variété de l'igname *D. alata* à chair pourpre. Tous les 3 sont des glycosides de la cyanidine: le pigment principal est le cyanidine-3, 5-diglucoside.

V. RAŠPER and D. G. COURSEY[13]

Department of Biochemistry, Nutrition and Food Science, University of Ghana, Legon, Accra (Ghana), 27th February 1967.

---

[9] W. G. C. FORSYTH, Biochem. J. *51*, 511 (1952).

[10] M. A. JERMYN and S. A. ISHERWOOD, Biochem J. *44*, 402 (1949).

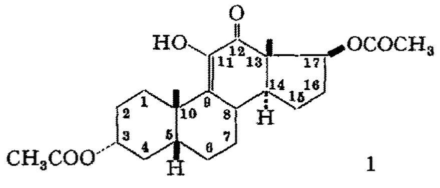
[11] E. Merck AG, *Chromatography*, Darmstadt, p. 135.

[12] J. B. HARBORNE and H. S. A. SHERRATT, Experientia 13, 486 (1957).

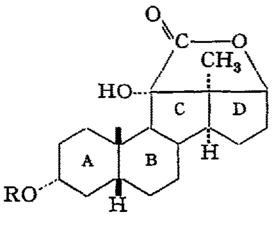
[13] Present address: Tropical Products Institute, 56/62 Gray's Inn Road, London, W.C.1 (England).

The Molecular Structure of the Benzilic Acid Rearrangement Product of 3α,17β-Diacetoxy-11-hydroxy-12-oxo-5β-androst-9(11)-ene

3α, 17β - Diacetoxy - 11 - hydroxy - 12-oxo - 5β - androst - 9(11)-ene (1), on treatment with base, gives a compound $C_{19}H_{28}O_4$, which can be shown to contain 2 hydroxyl groups and a lactone ring[1]. A benzilic acid rearrangement of 1, followed by lactonization between the carboxyl group at C-11 and the hydroxyl at C-17 would be stereochemically impossible with a *trans* C/D ring junction. Accordingly, a retroaldol equilibrium with epimerization at C-13 leading to a *cis* C/D ring junction prior to the benzilic acid rearrangement was postulated, and structure 2a was put forward for the lactone[1]. In this remarkable transformation numerous keto-enol equilibria could lead to a change of stereochemistry of the B/C as well as the C/D ring junctions. A crystal structure analysis was carried out on a *p*-bromobenzoyl ester of the rearrangement product to determine the stereochemistry at the B/C ring junction, postulated to be that of the naturally occurring androstanes, and to confirm the unusual structure containing three 5-membered rings all *cis*-fused[1].



1



2a R = H
b R = p-BrC_6H_4CO

[1] P. KURATH, Experientia *22*, 657 (1966).