THE CHARACTERISATION OF CRYSTALLINE AND AMORPHOUS ALOIN

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Tests are described showing the differences between amorphous aloin and that of the B.P. 1953. One of these tests might, with advantage, be incorporated in official publications to differentiate crystalline aloin from the relatively impure and less active amorphous aloin.

AN amorphous aloin which does not conform to the description in the British Pharmacopoeia, 1953, has appeared in commerce, particularly in the export market. Aloin is described as "a pale yellow, microcrystalline powder"; the amorphous material shows no trace of crystallinity under the polarising microscope. Other samples appeared to contain a mixture of amorphous and crystalline material.

Crystalline aloin of the Pharmacopoeia is obtained mainly from Cape and Curaçao aloes by a process involving precipitation of the calcium salt, subsequently decomposed by hydrochloric acid. The product consists of small yellow crystals, soluble in about 100 parts of water which contain a high proportion of barbaloin[1,2]; the yield is about 12 per cent from Cape and 25 per cent from Curaçao aloes.

Amorphous aloin is obtained in about 60 per cent yield from a dried extract of Cape aloes, free from resinous material, by solvent extraction. The sample thus prepared was compared with a sample of German origin. Both were yellow powders, low in barbaloin content[1] and very soluble in water; both turned rapidly to a sticky paste with a few drops of water. This simple test will distinguish amorphous aloin from that conforming to the B.P., 1953, standard, which remains mostly solid when a small quantity of water is added. The two amorphous samples were identical, as shown by ultra-violet spectrophotometry and paper chromatography.

The catharsis produced by a German sample of amorphous aloin was investigated by Auterhoff and Ball[2]. Tests on human subjects indicated an effective dose for amorphous aloin more than double that for crystalline aloin, and about two thirds that for Cape aloes. A satisfactory method of bioassay for aloes extracts using rats was described[3], and this was used by us.

EXPERIMENTAL

The following materials were used in the experiments: Crystalline aloin (B.P. 1953), amorphous aloin, amorphous aloin (German), barbaloin, purified as described by Hay and Haynes[4], and Cape aloes, powdered.

Ultra-violet Absorption Spectra

The curves (Fig. 1) were obtained with aqueous solutions, concentration 0·0025 per cent w/v, in 1-cm. quartz cells on a Unicam S.P. 500 spectrophotometer. The spectra of the amorphous samples were identical, with maxima at 254 and 298 mμ [E (1 per cent, 1 cm.) values of about 300 and 320, respectively]. The spectrum of aloin, B.P., resembled that of barbaloin, differing only slightly in intensity at the maxima at 269, 298 and 354 mμ [E (1 per cent, 1 cm.) values 190, 225 and 245 for aloin and 215, 240 and 260 for barbaloin, respectively]. The spectrum of aloin, B.P., thus shows a maximum (at 354 mμ) absent from that of the amorphous material. Measurement of the relative intensities at 354 and 298 mμ provides a convenient means for distinguishing crystalline aloin from amorphous.

Absorption spectra previously reported for crystalline aloin[1,5] and for German amorphous aloin[1] were similar to those of Figure 1.

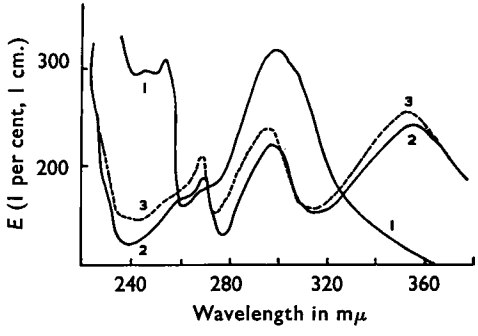

FIG. 1. Ultra-violet absorption spectra. 1. Amorphous aloins. 2. Aloin B.P. 3. Barbaloin.

Paper Chromatography

Several workers have investigated the separation of aloes constituents by paper chromatography; most of them favoured a solvent mixture comprising *n*-butanol, acetic acid and water[1,6–8]. This mixture appeared to have no advantage over *n*-butanol saturated with water which we used. Spots of aqueous solutions, of the following concentrations, were applied at the starting line on sheets of Whatman No. 2 paper. Amorphous aloin (German), 0·4 per cent w/v; barbaloin, 0·2 per cent w/v; amorphous aloin, 0·4 per cent w/v; aloin, B.P., 0·25 per cent w/v, and Cape aloes, 2·0 per cent w/v.

Development was by the ascending method for 30 hours at 20° $\pm$ 1·5. When the paper was dried, and examined under ultra-violet illumination from a Hanovia "Chromatolite", the spots were seen to be clearly separated. Every sample on the paper showed a spot with dark orange fluorescence changing to bright yellow in contact with ammonia vapour. This spot proved to be barbaloin (R_F about 0·77). Crystalline aloin (B.P. 1953) showed the barbaloin spot only. In both the samples of amorphous aloin and in the sample of Cape aloes, the yellow barbaloin spot was between two bright blue fluorescent spots (R_F values about 0·57 and 0·87). Similar spots observed by previous authors were reported as *p*-cumaric acid and an unknown anthracene derivative[8].

The chromatograms were also photographed on reflex-copying paper by an ultra-violet printing technique similar to that of Markham for nucleic acids[9]. Negatives obtained in this way were re-printed as positives

in a photo-copier. The enhanced contrast of the final prints rendered the spots clearly visible. The two amorphous aloin samples were seen to be alike, and to have the same principal constituents as the water-soluble fraction of Cape aloes.

Assay of Aloin

No satisfactory chemical method of assay is known for aloes preparations (see, however, ref. 1). Consignments of aloes are usually assessed by the isolation of aloin. Typical recoveries of aloin from Cape aloes are 12 to 15 per cent. The procedure was applied to amorphous aloin and to aloin, B.P., as a further means of distinction; the yields obtained were markedly different.

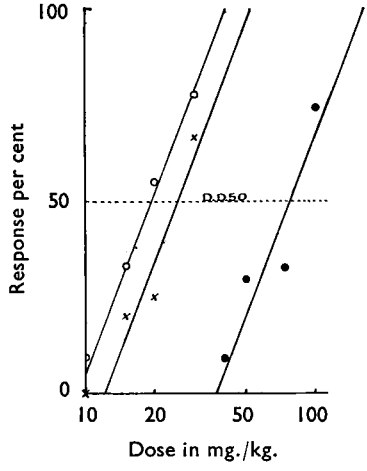

FIG. 2. Dose response curves for three samples of aloin. ○—○, Barbaloin. ×—× Aloin, B.P. 1953. ●—●, Amorphous aloin.

Amorphous aloin (5 g.) was dissolved in 350 ml. of warm, dilute hydrochloric acid (pH about 3·0). The insoluble residue was filtered and washed with a further 50-ml. portion of the same acid. Filtrate and washings were combined and cooled to room temperature. Calcium hydroxide (1·25 g.) was added and the mixture well stirred. Hydrochloric acid was added until the solution was just moderately alkaline, and the mixture was stirred for 90 minutes. The precipitate was then filtered in a Buchner funnel, sucked dry, and washed with two 10-ml. portions of ice-water. It was then transferred (as rapidly as possible) to a small beaker where it was mixed with concentrated hydrochloric acid (2·5 ml.). The mixture was gently warmed until a clear solution was obtained. This was left overnight in the refrigerator. The product was filtered in a sinter-glass crucible, sucked dry, and washed with three 2-ml. portions of ice-water. It was then dried to constant weight in an oven at about 60°. The yield of crystalline aloin was 16 per cent, this low value being explicable in terms of the solubility of the calcium aloin. In a similar experiment with aloin, B.P., the yield was 91 per cent.

Bioassay

Barbaloin, aloin, B.P., and amorphous aloin were assayed by the method of Latven and others[3] for aloes extracts. This is based on an assessment of the consistency of rat faecal pellets. Oral administration of aloin to rats produces catharsis characterised by light-coloured, soft, wet faecal pellets. This catharsis reaches a maximum at about 12 hours and persists for a further 12 hours; for convenience the assessment was made 17 hours after the animals were dosed.

The faecal consistency was assessed by allowing a faecal pellet to fall on to a clean glass plate; a hypodermic needle of 0·9 mm. diameter was inserted vertically downwards into the pellet and lifted. If the pellet adhered to the plate it was scored positive, if it was lifted by the needle (as was a normal pellet from an untreated animal) it was scored negative. Four groups of four female rats in the weight range 140 to 190 g. were used to test each sample.

The median defaecatory dose (DD50) for each sample was determined as described in the original paper[3]. When the percentage response was plotted against log-dose, straight lines were obtained (Fig. 2). These

TABLE I

THE DD50 OF ALOIN PREPARATIONS ADMINISTERED ORALLY TO FEMALE RATS

Preparation	Moisture content percentage w/w	DD50 and limits of error P = 0·95	Relative potency (after correction for moisture content)
Barbaloin	0	19·5 (15·3–25·0)	100
Aloin (B.P. 1953)	6	25·3 (17·3–37·0)	82
Amorphous aloin	8	78·2 (58·9–103·8)	27

lines showed no significant deviation from parallelism, which indicated that the three samples were acting in a similar manner.

The DD50 for each of the three samples, and their relative potencies after correction for moisture content, are given in Table I.

DISCUSSION

Sufficient evidence has been presented to show the marked differences between amorphous aloin and aloin as described in the British Pharmacopoeia, 1953. The first point of difference is, obviously, the amorphous nature of the material. The second is the high water-solubility. The two forms can further be distinguished by differences in the ultra-violet absorption spectra or paper chromatograms. The same criteria emphasize the close similarity of the amorphous material and the corresponding sample of German origin.

It is thus demonstrated that the amorphous type of aloin is a heterogeneous substance, low in barbaloin content, comprising the main water-soluble constituents of Cape aloes. Biological assay has shown that the potency of the amorphous aloin is about one third that of aloin, B.P.

Conclusions. It would be desirable to include a more specific test for crystalline aloin in official publications in view of the appearance in commerce of amorphous material, of different constitution, and lower biological potency. A suitable test involves measurement of the ratios of absorption intensities at 354 and 298 mμ: for crystalline aloin the intensity at 354 mμ is greater than that at 298 mμ; amorphous aloin, however, shows no maximum in the region of 354 mμ, where the intensity is less than that at 298 mμ.

The following description, therefore, specifies crystalline aloin:

Identification: A 1-cm. layer of a 0·0025 per cent w/v solution in water exhibits characteristic light absorption with maxima at 269, 298 and 354 mμ.

Light absorption: The extinction of a 1-cm. layer of a 0·0025 per cent w/v solution (freshly prepared) in water, calculated for the anhydrous material, at 298 mμ is about 0·55 and at 354 mμ about 0·61; the ratio of the extinction at 354 mμ to that at 298 mμ is greater than 1·0.

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After Dr. Lister presented the paper there was a DISCUSSION. The following point was made.

A microscopical means of differentiating between crystalline and amorphous aloin was to mount the material in cresol, when the amorphous material was soluble while the crystalline was not.