THE QUANTITATIVE CONVERSION OF BARBALOIN TO ALOE-EMODIN AND ITS APPLICATION TO THE EVALUATION OF ALOES

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Barbaloin can be quantitatively converted into aloe-emodin by heating a solution in 4 N hydrochloric acid, containing 4 per cent ferric chloride, for 4 hr. in a boiling water bath under reflux. Each of these numerical conditions can be varied ±10 per cent without significantly altering the yields. The reaction has been successfully applied to the assay of commercial samples of aloes.

BARBALOIN, 10(1)-deoxyglucosyl aloe-emodin anthrone, is an important constituent of aloes and has recently been shown to occur in cascara (*Rhamnus purshiana* D.C. bark) in the form of glycosides (Fairbairn and Simic, 1960). A method of determining the barbaloin content of these crude drugs would therefore be useful in their evaluation.

Numerous attempts have been made to determine barbaloin in aloes and its preparations. Of published methods (Brody, Voigt and Maher, 1950; Mary, Christiensen and Beal, 1956; Borkowski, Henneberg and Urszulak, 1960; Janiak and Böhmert, 1962; Kraus, 1957; Paris and Durand, 1956; Lister and Pride, 1959) none was considered satisfactory, mainly because of interfering substances in the crude drugs, especially in cascara. The conversion of barbaloin to aloe-emodin offered an alternative since aloe-emodin may be readily separated from impurities and estimated colorimetrically. Various reagents have been described for this conversion and of these we have found ferric chloride the most satisfactory (cf. Cahn and Simonsen, 1932; Harders, 1949; Hay and Haynes, 1956; Betts, 1961; Paris and Durand, 1956; Hörhammer, Wagner and Föcking, 1959). The recommended method is given in the experimental section. The results obtained on standard solutions of barbaloin are given in Table I.

In aloes, small quantities of free anthraquinones and normal glycosides are present; these are removed by the procedure described in the first paragraph in the section on "Estimation of the Barbaloin Content of Aloes".

Several authors have claimed that when extracts of aloe are chromatographed, a spot of R_F value less than barbaloin may be observed (Awe, Auterhoff and Wachsmuth-Melm, 1954; Awe and Kümmell, 1960). Unlike barbaloin, this spot, when treated with alkali, fluoresces blue in ultra-violet light: it has been suggested that the substance responsible is an isomer of barbaloin. Janiak and Böhmert (1962) claim it can be removed from aloe solution using a column of polyamide, but we have found different samples of polyamide to differ in their capacity to achieve this separation. Using paper chromatography, we separated some of

this material which, on treatment with ferric chloride, gave only a faint pink colour with sodium hydroxide in contrast to the deep red colour of the simultaneously eluted barbaloin. The faint colour may well be due to traces of barbaloin incompletely separated from the blue fluorescent substances. It did not interfere in our assay. Table II shows the results of applying our method to 5 commercial samples of curaçao aloes: the results are compared with those obtained by the calcium precipitation method (Lister and Pride, 1959).

EXPERIMENTAL

Materials

Barbaloin, prepared by the method of Hay and Haynes (1956), was crystallised from water then methanol in lemon yellow needles. When dried to constant weight, at 110 to 120° over magnesium perchlorate, it lost 4·6 per cent (mean of 4 analyses). On exposure to air the dried material very rapidly recovered its moisture content; after 15 min. 3·7 per cent gain; after 30 min. 4·4 per cent gain.

Found (anhydrous material) C, 60·5, 60·0; H, 5·3, 5·3. Calculated for $C_{21}H_{22}O_9$; C, 60·3; H, 5·3. Found (before drying): mean of 5 analyses; C, 57·6; H, 5·6. Calculated for $C_{21}H_{22}O_9$, H_2O: C, 57·8; H, 5·5; H_2O, 4·1 per cent. For the anhydrous material, m.p. 148–149° (lit. (Hay and Haynes, 1956) gives m.p. 148–148·5°); λ_{max} 269, 296·5 and 354 mμ; (*E* (1 per cent, 1 cm.) 192, 226 and 259 respectively).

TABLE I

YIELDS OF ALOE-EMODIN (MG./100 MG. BARBALOIN) USING THREE SOLUTIONS OF BARBALOIN. EACH SOLUTION WAS ASSAYED 4 TO 6 TIMES BY THE METHOD DESCRIBED

Solution 1 27·6 mg. barbaloin/litre	Solution 2 27·8 mg. barbaloin/litre	Solution 3 62·4 mg. barbaloin/litre
61·6	63·1	62·4
60·7	62·8	61·4
61·0	62·8	62·0
60·5	62·0	61·0
61·8		
61·0	Mean 62·7	Mean 61·7
Mean 61·1		

Grand Mean = 61·8 (s.d., 0·84).
100 per cent theoretical yield ≡ 61·9 (100 × $C_{15}H_{10}O_5/C_{21}H_{22}O_9$, H_2O).

Aloe-emodin, prepared by the method of Cahn and Simonsen (1932) was sublimed at 150° and 0·001/mm. Hg and crystallised from glacial acetic acid as orange needles, m.p. 225–226°, λ_{max} (N sodium hydroxide) 500 mμ; [*E* (1 per cent, 1 cm.); 320; at λ 440 mμ; 142].

Conversion of barbaloin to aloe-emodin. The following details represent the optimum conditions* for the hydrolysis. Barbaloin dissolved in 4N hydrochloric acid containing 4 per cent ferric chloride is heated at 100° for 4 hr. in a boiling water-bath under reflux. The cooled solution

* The amount of ferric chloride, HCl and the time can be varied ±10 per cent without altering the yields.

was then extracted with carbon tetrachloride† and assayed colorimetrically as described in the method. The yield of aloe-emodin from the barbaloin monohydrate (mean of 14 results, Table I) was 61·8 mg./100 mg. (theoretical yield, 61·9 mg.) and from the anhydrous barbaloin (mean of two results) was 63·6 mg./100 mg. (theoretical yield, 64·6 mg.).

Ammonium persulphate (Seel, 1919) and sodium metaperiodate (Hay and Haynes, 1956) gave variable results ranging from 0 to 35 mg./100 mg. barbaloin.

TABLE II

BARBALOIN CONTENT (PER CENT AIR-DRY MATERIAL) OF COMMERCIAL SAMPLES OF CURAÇAO ALOES

Sample	Present method	Ca precipitation method
1	30·1, 28·4, 29·4 } 29·3	22·5
2	31·5, 31·1 } 31·3	23·5
3	32·5, 33·5 } 33·0	22·9
4	33·3, 32·8 } 33·1	21·0
5	33·5, 33·1 } 33·3	22·6

Estimation of the Barbaloin Content of Aloes

Transfer about 0·2 g. powdered aloe sample, accurately weighed, to a 200 ml. flask, moisten with 2 ml. methanol, add 80 ml. hot water and shake for 30 min. Cool, filter into a 100 ml. volumetric flask and make up to volume. To 10 ml. of this solution add 1 ml. hydrochloric acid B.P. and heat for 15 min. in a boiling water-bath. Cool, extract with 2 × 20 ml. carbon tetrachloride, wash the combined tetrachloride layers with 2 × 10 ml. water, discard the carbon tetrachloride layer and return the washings to the aqueous acid layer; transfer this to a 100 ml. volumetric flask and make up to volume.

To 10 ml. of this solution add 6 ml. hydrochloric acid B.P. and 0·6 g. anhydrous ferric chloride and heat in a boiling water-bath under reflux for 4 hr. Cool, extract with 3 × 20 ml. carbon tetrachloride and wash the combined carbon tetrachloride extracts with 2 × 10 ml. water. Reject the washings. Extract the carbon tetrachloride layer with 15, 5 and 5 ml. of N sodium hydroxide; heat the combined alkaline extracts in a boiling water bath for 5 min. (to drive off traces of carbon tetrachloride), cool and make up to 25 ml. Determine the extinction of this solution, at 500 mμ within 1 hr. and estimate the concentration of aloe-emodin from the *E* (1 per cent, 1 cm.) value of 320 or from a suitable calibration curve. Calculate the percentage of barbaloin present from the fact that 1 mg. aloe-emodin is equivalent to 1·61 mg. $C_{21}H_{22}O_9 \cdot H_2O$.

† Carbon tetrachloride is a specific solvent for aloe-emodin and is thus preferable to the ether or butanol used by other workers (Auterhoff and Ball, 1954; Harders, 1949; and cf. Fairbairn, 1942).

The absorption curve of this alkaline solution between 440 and 550 mμ was in close agreement with the curve obtained from a sample of pure aloe-emodin in an alkaline solution of the same concentration.

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REFERENCES

Auterhoff, H. and Ball, B. (1954). *Arzneimitt.-Forsch.*, **4**, 725–729.
Awe, W., Auterhoff, H. and Wachsmuth-Melm, C. L. (1958). *Ibid.*, **8**, 243–245.
Awe, W. and Kümmell, H. J. (1960). *Arch. Pharm., Berl.*, **293**, 271–277.
Betts, T. J. (1961). Thesis, p. 105, University of London.
Borkowski, B., Henneberg, M. and Urszulak, I. (1960). *Biul. Inst. Rosl. Leczn.*, **6**, 125–135.
Brody, T. M., Voigt, R. F. and Maher, F. T. (1950). *J. Amer. pharm. Ass. Sci. Ed.*, **39**, 666–672.
Cahn, R. S. and Simonsen, J. L. (1932). *J. chem. Soc.*, 2573–2582.
Fairbairn, J. W. (1942). *Pharm. J.*, **148**, 198–199.
Fairbairn, J. W. and Simic, S. (1960). *J. Pharm. Pharmacol.*, **12**, 45*T*–51*T*.
Harders, C. L. (1949). *Pharm. Weekbl.*, **84**, 250–258, 273–281.
Hörhammer, L., Wagner, H. and Föcking, O. (1959). *Pharm. Ztg., Berl.*, **104**, 1183–1186.
Hay, J. E. and Haynes, L. J. (1956). *J. chem. Soc.*, 3141–3147.
Janiak, B. and Böhmert, H. (1962). *Arzneimitt.-Forsch.*, **12**, 431–435.
Kraus, L. (1957). *Pharmazie*, **12**, 693–695.
Lister, R. E. and Pride, R. R. A. (1959). *J. Pharm. Pharmacol.*, **11**, 278*T*–282*T*.
Mary, N. Y., Christiensen, B. V. and Beal, J. L. (1956). *J. Amer. pharm. Ass. Sci. Ed.*, **45**, 229–232.
Paris, R. and Durant, M. (1956). *Ann. pharm. franç.*, **14**, 755–761.
Seel, E. (1919). *Arch. Pharm., Berl.*, **257**, 212–259.