Phytochemistry, 1965, Vol. 4, pp. 639 to 641. Pergamon Press Ltd. Printed in England

SHORT COMMUNICATION

DIFFERENTIATION BETWEEN *C*- AND *O*-GLYCOSYL COMPOUNDS

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(*Received* 15 *November* 1964)

Abstract—A method for distinguishing between *C*-glycosyl compounds and *O*-glycosides depending on the acid stability of the borohydride reduction products of periodate-oxidized compounds[1] has been examined and found to give equivocal results. The method can, however, be of value in determining the structure of the glycosyl residue. Examination of NMR spectra and the products of methylation have established the *C*-glycosyl nature of orientin and iso-orientin.

THE recognition of compounds as *C*-glycosyl derivatives is usually based on the fact that they fail to liberate a sugar on hydrolysis even under prolonged and fairly drastic acidic conditions. However, as pointed out by Haynes,[1] such results should be interpreted with caution since several 7-glycosyloxyflavones and flavonols require quite vigorous conditions for hydrolysis whereas some *C*-glycosyl compounds such as vitexin and D-glucopyranosylbenzene will liberate glucose under relatively moderate conditions such as by heating with 10% sulphuric acid at 120° for 4 hr.

Hay and Haynes[2] have suggested a method for differentiating between *C*- and *O*-glycosyl compounds which depends on the fact that the products formed by periodate oxidation and subsequent borohydride reduction from *C*-glycosides are ethers which would be more stable to acidic hydrolysis than the corresponding products from the *O*-glycosides which are acetals. Thus, for hexopyranosyl compounds, only the product from an *O*-glycoside should yield glycerol on hydrolysis and the failure to detect glycerol on acid treatment of the product from barbaloin was therefore taken as additional evidence of the *C*-glycosyl nature of this compound.[2] Haynes[1] considers that no *C*-glycoside yields glycerol under such mild, acidic conditions unless special structural features cause the ether linkage to be unstable. Such is the case with bergenin where a base-catalysed elimination of glyceraldehyde from periodate-oxidized di-*O*-methylbergenin occurs in sodium borohydride solution, with subsequent reduction of the glyceraldehyde to glycerol by the reagent.[3] Careful reduction of periodate-oxidized di-*O*-methylbergenin in buffered borohydride solution is, however, reported to have resulted in the formation of a product which did not undergo hydrolysis to yield glycerol on subsequent treatment with acid.[3]

In spite of the above considerations, glycerol has been reported as a product of periodate oxidation, borohydride reduction and acid hydrolysis of iso-orientin[4] (formerly known as homo-orientin), of orientin and iso-orientin tetramethyl ethers[5] and of barbaloin.[5] As it is

[1] L. J. HAYNES, *Advances in Carbohydrate Chem.* **18**, 227 (1963).
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[4] R. HÄNSEL and H. RIMPLER, *Arch. Pharm.* **296**, 598 (1963).
[5] B. H. KOEPPEN, *Z. Naturforsch.* **19b**, 173 (1964).

inherently unlikely that cleavage of the ethereal bond of the periodate-oxidized compounds could have occurred during the alkaline conditions of borohydride reduction (a possibility which has now been eliminated by experiment), it is evident that this bond is not insensitive to cleavage by acid under fairly mild conditions.[4,5] These conditions were, however, rather more strongly acidic than those previously employed for barbaloin[2] and the possibility was also considered that the negative glycerol test reported for orientin[6] might have been due to hydrolysis under conditions of very low acidity as the actual acid concentration employed by these workers[6] was not specified.

It therefore appeared desirable to examine more carefully the acid stability of the products of periodate oxidation and borohydride reduction of various *C*-glycosyl compounds. Previous workers[2,4,6] have effected these oxidations and reductions by the micromethod of Viscontini, Hoch and Karrer[7] but in the present study a slightly more elaborate and larger scale method was employed in order to control the reaction conditions more carefully. The compounds studied included barbaloin and the tetramethyl ethers of orientin and iso-orientin. In all cases, hydrolysis of the reduced oxidation products in 0·1 N-hydrochloric acid at 100° for 5 min (conditions considerably milder than those employed by Hay and Haynes for barbaloin[2]) yielded glycerol in amounts comparable to those from equivalent quantities of methyl α-D-glucopyranoside when treated similarly. The main value of this technique therefore appears to be in determining the ring size of glycosyl residues.

The true *C*-glycosyl nature of barbaloin has been unequivocally established[2] and the compound has also been synthesized.[8] The *C*-glycosyl nature of orientin is established by the following considerations. (1) The compound forms a tetramethyl ether under conditions which are restricted to the methylation of phenolic hydroxyl groups.[9] Thus, in the parent compound, the 3′-, 4′-, 5- and 7-hydroxyls of the luteolin nucleus must all be free. (2) In the NMR spectra of flavones with phloroglucinol-derived A-rings, the 6- and 8-protons appear upfield from the B-ring protons and are readily recognizable as a pair of doublets (e.g. chrysin[10] and apigenin[11]) with $J_{6,8} = 2{\cdot}5$ c/s owing to *meta*-coupling. The NMR spectrum of orientin shows the presence of only one A-ring proton and this appears as a simple, unsplit signal thus confirming that the position *meta* to this proton is substituted. (Similar results have been reported for vitexin[10,11] and iso-vitexin[11].)

Similar considerations apply to iso-orientin thus doubly establishing the *C*-glycosyl nature of these compounds as orientin and iso-orientin have been shown to be interconvertible.[6,9,12] Furthermore, this has been shown to be due to a Wessely-Moser rearrangement[5] so that the phloroglucinol-derived A-ring must be unsymmetrically substituted in each compound.

EXPERIMENTAL

Orientin and iso-orientin tetramethyl ethers were prepared as previously described.[9] Barbaloin was prepared from commercial aloin by recrystallization to constant m.p. (146–148°).

The compound (0·02 m-mole) was dissolved in aqueous ethanol (15 ml) and treated in the

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dark with aqueous periodic acid (0·04 N; 15 ml) for 24 hr at 15°. Aqueous barium hydroxide solution (5·6% w/v; 2 ml) was added and after 1 hr at 0° the precipitate was removed by centrifugation. The precipitate was washed with ethanol, centrifuged, and the supernatant and washings were combined. The solution was adjusted to pH 7·0 with dilute H_2SO_4 and filtered. The precipitate was washed with ethanol and sodium borohydride (15 mg) was added to the combined filtrate and washings. The mixture was kept overnight at 0° and the pH was readjusted to 7·0 with dilute HCl. The neutral solution was taken to dryness under reduced pressure at 55° and the residue was dissolved in ethanol (4 ml).

Examination of the ethanolic solution for the presence of glycerol by paper chromatography in butan-1-ol:acetic acid:water (20:5:11, v/v) showed, in all cases, no trace of this compound on spraying with sodium *meta*periodate and benzidine solutions.[2]

The acid stability of the periodate-oxidized, borohydride-reduced compounds was examined as follows. Hydrochloric acid (0·02 N; 1 ml) was added to ethanolic solutions of the compound (1 ml) and the mixtures were immersed in a boiling water bath for 5, 10 or 15 min under reflux. The hydrolysates were neutralized with dilute NaOH, desalted in a Shandon electrolytic desalter (Mark II), concentrated to near dryness and examined by paper chromatography. Glycerol was readily detected in all cases.