Short Communication

THE STRUCTURE OF CASCAROSIDES A AND B

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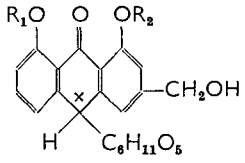
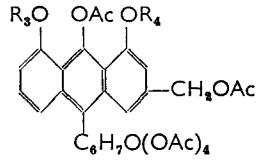
A previous communication (Fairbairn and Simic, 1960) reported the isolation and properties of two substances, cascaroside A and cascaroside B, obtained from Cascara (*Rhamnus purshiana* DC bark). Their general properties, ultra-violet light absorption curves and behaviour on chromatograms were similar, but a significant difference was noted in their optical rotations. The further work reported in this present paper is concerned with the relationship between cascarosides A and B, the products of hydrolysis other than barbaloin and the position of attachment of these groups to barbaloin.

The Relationship between Cascaroside A and B

Since the infra-red spectra of cascarosides A and B were almost identical, it seemed likely that they were stereoisomeric since no significant structural differences could be deduced from the curves. The nature of the isomerism was established by acetylation of the two cascarosides with acetic anhydride in pyridine when two anthranol acetates were obtained which were identical in optical rotation ($[\alpha]_D^{20°} = -51°$) and melting point with no depression of the latter on admixture. They can be represented by part structure (II) in which the tetrahedral arrangement at C_{10} responsible for the isomerism of the cascarosides has now become planar.



I. *Cascarosides*
R_1, R_2 = H or glycone



II. *Acetyl cascaroside*
R_3, R_4 = acetyl or acetyl glycone

The isomerism of the cascarosides is therefore attributable to the aglycone moiety and not to the nature or position of attachment of the glycones. This was confirmed by hydrolysis of cascarosides A and B in N hydrochloric acid at 70° under nitrogen when two different barbaloins (the aglycones) were obtained. Their melting points, R_F values in three different systems and reactions to the usual barbaloin tests (including the cupraloin test) were identical. They differed slightly in crystalline form; aglycone A readily formed yellow needles whilst aglycone B crystallised with difficulty and tended to form clusters of fine needles. The main

difference however was in optical rotation: the $[\alpha]_D^{20}$ in methanol (*c*, 2·5) for aglycone A was +7·60° and for aglycone B was −47·8°. Cascaroside A is therefore a glycoside of (+)-barbaloin and cascaroside B of (−)-barbaloin. These two isomers of barbaloin may be barbaloin and iso-barbaloin respectively which, Mühlemann (1952) suggests, are possibly optical isomers.

The Glycones

Glucose was shown to be present in the hydrolysate of both cascarosides. It was identified by its R_F value and by its infra-red spectrum. Quantitatively, the cascarosides yielded 56 to 60 per cent barbaloin and about 40 per cent by weight of glycone. Of this only about half was glucose; the rest was a non-reducing glycone. The presence of two glycones was confirmed by methanolysis of the cascarosides using methanol in the presence of ZeoKarb 225+ resin. Paper chromatography showed 1-α-methyl-D-glucose ($R_F = 0{\cdot}17$) and a second non-reducing component ($R_F = 0{\cdot}03$) revealed as a yellow spot on exposure to iodine vapour.

Both cascarosides undergo oxidation with aqueous sodium periodate at 0° with the consumption of 7 moles of reagent. Under these conditions barbaloin (and homonataloin, Haynes, Henderson and Tyler, 1960) requires 2 moles; hence the two glycones must consume 5 moles of periodate. If we assume that the glucose is attached to the barbaloin by a 1-glucosidal link (indicated by rapid acid or enzyme hydrolysis) then it will consume 2 moles of periodate, leaving 3 moles for the other glycone. This would then contain the part structure $(CHOH)_4$ and would probably be linked to the barbaloin by means of a further –C–O– group. Such a 5 carbon molecule would fit our previous analytical results which indicate that the proportion of this glycone present is less than that of glucose; that is, the molecular weight is less than 180.

Attachment of the Glycones to the Barbaloins

Since both cascarosides have identical acetyl derivatives, the points of attachment and conformation of the glycones must be similar in the two compounds. One possibility is that one glycone is attached to each of the two phenolic groups of the barbaloin and in this connection certain ultra-violet spectra are of interest. Hydrolysis of the cascarosides to barbaloin shifts the absorption maximum at 325 mμ to 354 mμ; conversely methylation of barbaloin to dimethyl-barbaloin (I; $R_1 = R_2 = Me$) shifts the maximum back to 325 mμ. Although this agrees with the suggestion that cascarosides, like dimethyl-barbaloin, have no free phenolic groups, their infra-red spectra indicate that both phenols are not blocked and that the shifts observed in ultra-violet light are probably due to phenomena more complex than phenol-ketone chelation. Thus the carbonyl group in the cascarosides, as in barbaloin, absorbs at 1636 $cm.^{-1}$ which is about 40 $cm.^{-1}$ lower than the unbonded quinone absorption. Since the carbonyl group in barbaloin is clearly bonded with its two free phenolic groups (cf. Haynes, Henderson and Taylor, 1960) it seems probable that the cascarosides contain at least one free phenolic

–OH group with which the carbonyl group is bonded. This is supported by the following observations:

(i) The ultra-violet absorption spectrum in alkaline solution produces a shift from 320 to 390 mμ. (In barbaloin the shift is from 354 to 388 mμ).

(ii) Both barbaloin and cascaroside in ethanolic solution give a deep green colour with ferric chloride (however sennoside, rheinanthrone-8-glucoside and rheinanthrone do not give colours with this reagent).

(iii) Both cascarosides and barbaloin give a yellow complex with methanolic magnesium acetate in which the peaks at 323 and 354 mμ have been shifted to 394 and 430 mμ respectively. The corresponding peaks in rheinanthrone glucoside and rheinanthrone shift from 340 to 380 and from 364 to 450 mμ respectively.

The spectroscopic results therefore indicate that there is, at least, one free phenolic group present. The two glycones may either be linked to each other and attached to one of the phenolic groups or one glycone may be so attached and the other attached to one of the alcoholic groups of the barbaloin.

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