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ASPALATHIN : A NOVEL C-GLYCOSYLFLAVONOID FROM ASPALATHUS LINEARIS.

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An amorphous polyphenolic compound, $C_{21}H_{24}O_{11}$, previously isolated from the leaves of Aspalathus linearis (1) (rooibos tea, formerly known as Aspalathus acuminatus) and named aspalathin (2) gives a crystalline nona-acetate, $C_{39}H_{42}O_{20}$, m.p. 152-154° and a non-crystalline pentamethyl ether, $C_{26}H_{34}O_{11}$, with methanolic diazomethane.

Alkali fusion of aspalathin results in degradation to phloroglucinol and protocatechuic acid. Treatment with aq. 2N hydrochloric acid fails to liberate any sugar, even after refluxing for 2 hr. and this result suggested that aspalathin, like the accompanying orientin (2) and vitexin, might be a C-glycosyl compound.

The compound has $\lambda_{max.}$ 290 mμ in ethanol, shifted to 308 mμ in ethanolic 75 mM aluminium chloride solution. This relatively large bathochromic shift, together with the ability of aspalathin to form a chelate with aluminium chloride which fluoresces a bright yellow-green on paper chromatograms under ultraviolet light, indicated the direct attachment of a

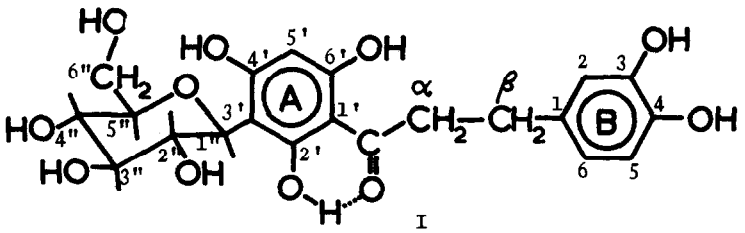
carbonyl group to the phloroglucinol nucleus (A-ring). The readiness with which the aluminium complex forms, suggested the occurrence of the carbonyl group in a six-membered heterocyclic ring or in an acyclic structure (3). Further evidence of the carbonyl group was provided by the presence of absorption bands at 1695 cm^{-1} and 1710 cm^{-1} in the infrared spectra of the penta-methyl ether and nona-acetate respectively. However, the colourless nature and ultraviolet spectrum of aspalathin preclude the catechol nucleus (B-ring) from also being conjugated with the carbonyl group (cf. 4).

One of the most significant reactions of aspalathin is its slow conversion in ethanolic solution in the presence of sunlight to a compound which gives a strongly positive sodium boro-hydride-aluminium chloride reaction on paper chromatograms. The compound is identical by chromatography with 2,3-dihydroiso-orientin, prepared by catalytic hydrogenation of iso-orientin with the aid of Adams' platinum oxide under conditions which yield eriodictyol from luteolin (5). Only traces of 2,3-dihydro-orientin can be detected during the initial stages of the photo-chemical reaction although the concentration of this flavanone increases with time owing to a Wessely-Moser rearrangement of the first-formed product. No photochemical conversion of aspalathin to flavanone takes place in the absence of oxygen and it therefore appeared that the compound was more highly re-duced than a flavanone. In view of these findings, a dihydro-chalcone structure for aspalathin appeared to be the most feasible even though phloretin was not converted to naringenin under similar photochemical conditions.

Periodic-acid oxidation of aspalathin pentamethyl ether

under conditions previously described (6) results in the uptake of two moles of periodate per mole of aspalathin with the formation of one mole of formic acid (calculations based on a C-hexosyldihydrochalcone structure). Borohydride reduction of the periodate-oxidized methyl ether and acid hydrolysis of the product results in the liberation of glycerol. Aspalathin therefore contains a hexopyranosyl residue which was finally established as a glucosyl residue by the detection of glucose and arabinose as products of ferric chloride oxidation of the compound or its methyl ether (cf. 7, 8).

The identity of aspalathin as a C-glucopyranosyldihydrochalcone for which structure (I) is preferred to the



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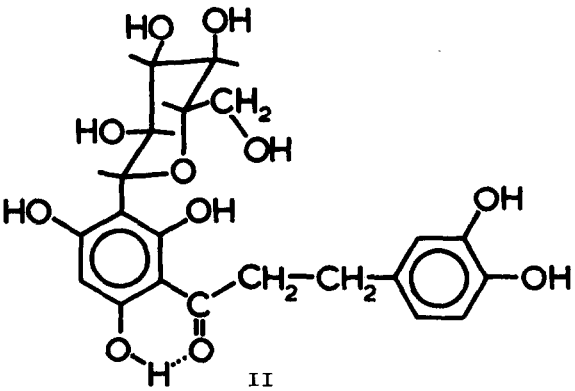

II

isomeric structure (II), is supported by n.m.r. studies (9).

The spectrum of aspalathin acetate exhibits a three-proton signal at τ 2.92 p.p.m. attributable to the protons of the B-ring and a one-proton singlet at τ 3.00 p.p.m. attributable to the single aromatic proton of the A-ring. The signals over the range τ 4.0 - 6.7 p.p.m. account for the seven protons of the glucosyl residue. One of these, a doublet centred at τ 5.23 p.p.m. is assigned to the C-1" proton, the large coupling constant (J, 10 c.p.s.) due to a trans-diaxial coupling with the C-2" proton indicating a β-C-glucopyranosyl residue (cf. 10). A signal centred at τ 6.98 p.p.m. indicated the presence of two methylene groups. A total of 27 protons is observed over the range τ 7.50 - 8.25 p.p.m. and in conformity with the analyses, these are attributable to the nine acetyl groups. One of these three-proton signals occurs significantly further upfield from the rest (τ 8.22 p.p.m.). This has been assigned to the C-2" acetyl (11) as this is the most likely group to be affected by the magnetic anisotropy of the A-ring. The diamagnetic shielding of the C-2" acetyl further requires this group to have an equatorial orientation and the spectrum therefore supports the identification of aspalathin as a C-β-D-glucopyranosyl compound.

Acetylation of aspalathin methyl ether yields an amorphous product, $C_{34}H_{42}O_{15}$, whose spectrum confirms the presence of five methoxyl and four acetyl groups. Again, a signal at τ 6.94 p.p.m. indicated the presence of two equally shielded methylene groups.

Treatment of aspalathin acetate with N-bromosuccinimide and benzoyl peroxide in chloroform (12) results in the formation of a crystalline monobromo derivative, $C_{39}H_{41}O_{20}Br$, m.p.

185°, which is considered to be the α-bromodihydrochalcone and the spectrum of the product confirms this. Thus, no signals in the region of τ 7.0 p.p.m. are present and the residual three protons of the ethylenic group appear as a characteristic A_2X system, the remaining proton of the α-C as a triplet (intensity 1:2:1) centred at τ 4.48 p.p.m. and the β-methylene protons as a doublet at τ 6.30 p.p.m.

Comparison of the spectra of aspalathin and phloretin in pyridine solution in the τ 6 - 7 p.p.m. region reveals a striking similarity in the pattern of the ethylenic protons in each compound. In each case, the protons on the α- and β-carbon atoms are resolved into two multiplets centred at τ 6.43 and 6.90 p.p.m. respectively in aspalathin and at τ 6.33 and 6.80 p.p.m. respectively in phloretin. The C-1" proton is also particularly clearly evident in the spectrum of aspalathin in this solvent (τ 4.30 p.p.m., J = 10 c.p.s.).

In the spectrum of aspalathin in dimethyl sulphoxide, a broad signal at τ -3.70 p.p.m. is observed and this can be assigned to the 2'-hydroxyl proton which is H-bonded to the carbonyl group. By comparison, phloretin exhibits a signal at τ - 2.30 p.p.m. due to the proton of the 2'-hydroxyl group (13). Methylation of aspalathin with the theoretical quantity of dimethyl sulphate to yield a tetramethyl ether results in the formation of a product which still has a positive ferric chloride reaction and whose spectrum in dimethyl sulphoxide still exhibits low field resonance due to an H-bonded phenolic hydroxyl proton (τ -4.00 p.p.m.). The introduction of a C-glycosyl residue in the 6- or 8-position of a 5-hydroxyflavone results in deshielding of the 5-hydroxyl proton (13),

the deshielding being greater for the o- than for the isomeric p-substituted compound (8,11). In view of this, the H-bond might be stronger in (I) than in (II) and this would account for the almost exclusive formation of 2,3-dihydroiso-orientin during the initial stages of photochemical oxidation.

Aspalathin, identified as 3'-C-β-D-glucopyranosyl-3,4,2',4',6'-pentahydroxydihydrochalcone, appears to be the first known example of a naturally occurring C-glycosyl-dihydrochalcone.

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