

Degradation of Condensed Tannins: Structure of the Tannin from Common Heather

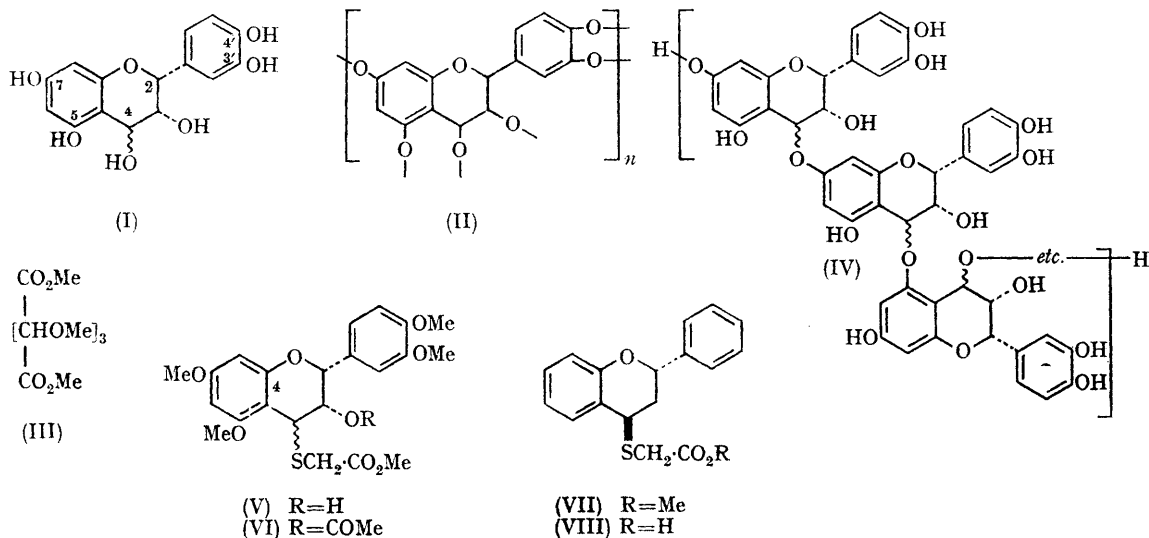
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SATISFACTORY methods are available for the isolation of some condensed vegetable tannins but degradative procedures for determination of their structures leave much to be desired.¹⁻³ We report two methods—ozonolysis and reaction with thiols—which have enabled a large part of the structure and stereochemistry of the tannin from common heather (*Calluna vulgaris*, Salisb.)¹ to be determined.

Production of about 5% cyanidin chloride by treatment of the tannin with concentrated hydrochloric acid and of phloroglucinol and protocatechuic acid by the action of steam showed that the flavanoid material in the tannin is hydroxylated at positions 5, 7, 3', and 4' [*e.g.*, (I)].¹ Analyses for C, H, N, inorganic residue, free phenolic groups with an unsubstituted *p*-position (Gibbs reagent⁴), and *o*-dihydric phenolic groups (ammonium molybdate⁵ and ferrous tartrate⁶ methods) are consistent with the view that the whole of the tannin is composed of flavanoid units, *viz.*, that it is polymerized 5,7,3',4'-tetrahydroxyflavan-3,4-diol (I). Ozonolysis⁷ of the

tannin confirmed this view: each C₁₅ unit in the tannin, calculated from C and H analyses, required after ozonolysis and treatment with hydrogen peroxide a titre of 10.07 moles of sodium hydroxide and 1.91 moles of potassium periodate [the partial structure (II) in yielding 4 moles of oxalic acid, 1 mole of trihydroxyglutaric acid, and 2 moles of carbon dioxide which is not titrated requires 10.0 moles of alkali and 2.00 moles of periodate]. Methylation of the reaction mixture yielded dimethyl trimethoxyglutarate (III) identified by comparison of infrared and n.m.r. spectra. This result indicates that in the tannin C-2, C-3, and C-4 of the flavanoid all carry oxygen substituents. The observation that the 3',4'-hydroxy-groups in ring B are free (ammonium molybdate and ferrous tartrate titres) whereas about half of those at C-5 in ring A are substituted, *i.e.*, are ethers (Gibbs reagent) and the assumption, reasonable mechanistically, that a benzylic hydroxy-group at C-4 is more likely to be involved in ether links than the relatively inert hydroxy-group at C-3 thus lead to a structure such as (IV) with C-4-O-C-5 and



C-4-O-C-7 ether links within the same molecule or to the view that the tannin is a mixture of molecules, some containing only C-4-O-C-5 links and others only C-4-O-C-7 links.

Such a structure should be capable of ready degradation by rupture of the benzylic C-4 ether links. To this end we have used thioglycolic acid, a reagent known to cleave benzylic ethers to *S*-benzylthioglycolic acids,⁸ to degrade the tannin into monomers. The crude product of this reaction was methylated by diazomethane, and purified by multiple-elution t.l.c., to give methyl *S*-(3-hydroxy-5,7,3',4'-tetramethoxyflavan-4-yl)-thioglycolate (V) as an amorphous powder, m.p. 45–50°, ν_{\max} (CHCl₃) 3590 m, 1735s cm⁻¹, λ_{\max} (EtOH) 276 m μ . The identification was based mainly on the n.m.r. spectra of the compound and of its amorphous acetate (VI), m.p. 40–45°, ν_{\max} (CHCl₃) 1738s cm⁻¹, λ_{\max} (EtOH) 276 m μ , which indicate 2,3-*cis*- and, less certainly, 3,4-*trans*-stereochemistry. This stereochemistry is that of the major isomer in the isolated product. It is likely that the 2,3-*cis*-3,4-*cis*-configuration is also present (and hindering crystallization), but in too low a concentration to be unambiguously detected in the n.m.r. spectra.

The mass spectra of the alcohol and its acetate gave molecular ions at 450 and 492 respectively, the calculated molecular weights. The major breakdown patterns were in each compound initiated by the loss of the C-4 sulphur substituent ($M - 105$ peaks) in accordance with the structures suggested.

Confirmation of the 2,3-*cis*- geometry was

obtained by treatment of the alcohol (V) with Raney nickel. This led to (–)-tetra-*O*-methyl-epicatechin, identical with a sample prepared from natural (–)-epicatechin, m.p. and mixed m.p. 141–143°, $[\alpha]_D^{20} - 56^\circ$, and gave further evidence that the sulphur substituent was at C-4 of the flavanoid nucleus.

The position of the sulphur substituent in the degradation product indicates that the ether links between the monomeric units of the tannin involve C-4, and thus confirms the structure (IV) for the tannin which can be regarded as a linear polymer of the diol (I). The extent of polymerization of (I) is not known; from the behaviour of the tannin, especially during paper chromatography,¹ it seems likely that though the tannin is homogeneous in the type of flavanoid unit it contains, it is heterogeneous in molecular weight.

The reaction between flavan-4 β -ol and thioglycolic acid was investigated as a model system for the degradation. Methyl *S*-flavan-4 α -ylthioglycolate (VII) was isolated from the methylated reaction mixture as an oil, from which by hydrolysis was obtained *S*-flavan-4 α -ylthioglycolic acid (VIII) as needles, m.p. 122–124°. The structure and stereochemistry were deduced from the n.m.r. spectrum of the ester. The reaction mechanism appears to be S_N1 since under identical conditions flavan-4 α -ol also gave the 4 α -flavanoid (VII). Nothing, therefore, can be deduced about the stereochemistry at C-4 in the tannin which may be pure or epimeric as in the degradation product.

The degradation reaction appears to have

considerable generality: condensed tannins isolated from *Picea pungens*, *Picea orientalis*, *Pinus strobus*, and *Pinus densiflora* were all degraded by thioglycollic acid, as indicated by the appearance

of mobile spots on thin-layer and paper chromatograms. Other thiols (toluene- α -thiol, butane-1-thiol, and 2-methylpropane-1-thiol) also degraded *Calluna* tannin in acid solution.

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