Novel Metabolites of Hexahydroxydiphenic Acid Esters (Ellagitannins) from *Carpinus japonica*

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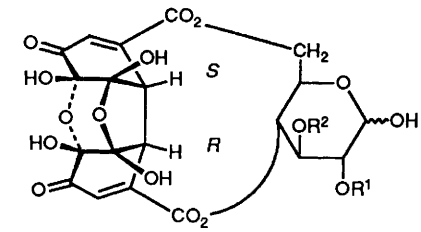
The unique hydrated biscyclohexenetrione structures (**1**)—(**4**) of carpinins A—D, the metabolites of hexahydroxydiphenic acid esters (ellagitannins) isolated from the leaves of *Carpinus japonica*, are described.

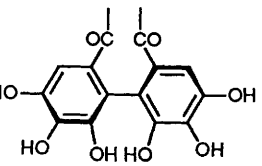
Recent chemical work on hydrolysable tannins has revealed that a variety of pathways exist in the metabolism of hexahydroxydiphenic acid esters (ellagitannins). These fall into two groups:[1] (i) oxidative coupling with adjacent galloyl ester groups leading to esters of much higher-molecular-weight phenolcarboxylic acids, such as flavaogallonic acid,[2] and (ii) oxidation of the aromatic rings into, *e.g.*, dehydrohexahydroxydiphenic acid.[3] In (ii), oxidation occurs invariably in one of the two aromatic rings at the C-2 and C-4 positions in the glucopyranose ring, which adopts a $^1C_4$ or the related skew-boat conformation.[4] We have now isolated from *Carpinus japonica* Blume (Betulaceae), a series of compounds (named carpinins A—D) (**1**)—(**4**), in which both of the aromatic rings in the hexahydroxydiphenoyl ester group are oxidised to a novel hydrated biscyclohexenetrione.

Carpinins A (**1**), B (**2**), C (**3**), and D (**4**),† were isolated in 0.007, 0.22, 0.01, and 0.009% yields (based on the fresh material), respectively, from an aqueous Me_2CO extract of fresh leaves by repeated chromatography on Sephadex LH-20 and various reverse-phase gels.[5] The ^{13}C NMR spectra of carpinins A—D all displayed signals due to carbonyl (δ *ca.* 192), tri-substituted alkene [δ *ca.* 130 (d) and 145 (s)], hemiketal (δ *ca.* 103 and 92), and benzylmethine (δ *ca.* 49) carbons, the chemical shifts being similar to those found in the dehydrohexahydroxydiphenoyl group.[4] Furthermore, the observation of a couple of α- (δ *ca.* 92) and β-anomeric (δ 95—98) signals indicated that these compounds exist in solution as mixtures of α- and β-anomers.

The presence of a hexahydroxydiphenoyl ester group in (**1**) and of two and one galloyl groups in (**2**) and (**3**), respectively, was suggested by their ^1H and ^{13}C NMR spectra. On heating in 3% HCl, (**1**) afforded (**4**), together with ellagic acid, while enzymatic hydrolysis of (**2**) and (**3**) with tannase yielded (**4**) and gallic acid. The formation of (**3**) from (**2**) was confirmed



(**1**) R^1, R^2 =



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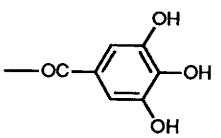
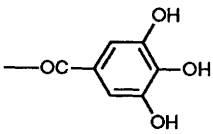
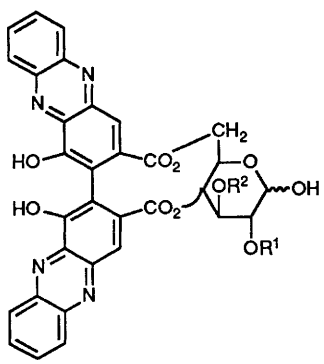


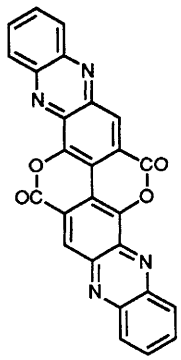
(**3**) R^1 = H, R^2 =

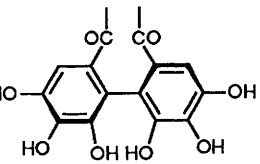


(**4**) $R^1 = R^2$ = H

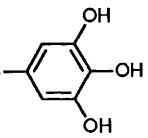
† Satisfactory analytical data (C, H) were obtained for all compounds described. *Selected data* for: (**1**), m.p. 245 °C (decomp.), $[\alpha]_D^{24}$ −39.8° (MeOH, *c* 0.9); (**2**), m.p. 252 °C (decomp.), $[\alpha]_D^{24}$ −9.6° (MeOH, *c* 0.9); (**3**), m.p. 254 °C (decomp.), $[\alpha]_D^{24}$ −81.8° (MeOH, *c* 1.0); (**4**), m.p. 266 °C (decomp.), $[\alpha]_D^{24}$ −7.6° (MeOH, *c* 0.9).

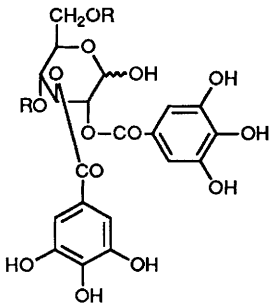


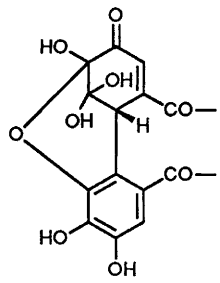


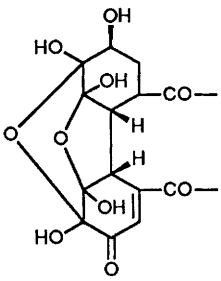
(5) R^1, R^2 = 

(7)

(6) $R^1 = R^2$ = 



(8) R = 

(9) R = 

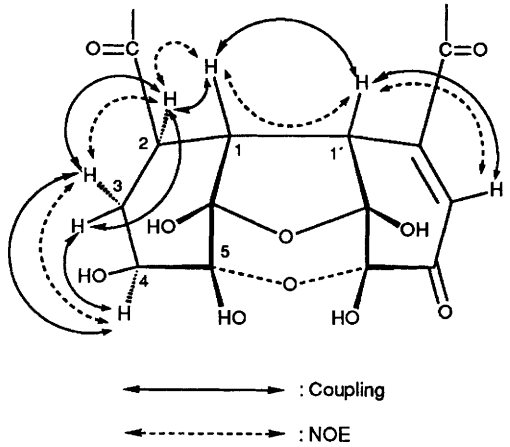


Figure 1. ^1H–^1H and NOE correlations in (**9**).

by its partial tannase hydrolysis. Thus, their mutual relationship was established. Condensation of (**1**) and (**2**) with *o*-phenylenediamine in the presence of HOAc[6] afforded the phenazine derivatives (**5**) and (**6**), which were subsequently heated in H_2O to give 2,3-(*S*)-hexahydroxydiphenoyl- and 2,3-di-*O*-galloyl-D-glucoses, respectively, together with the phenazine bislactone (**7**), confirming the locations of the acyl groups in (**1**) and (**2**). The galloyl group in (**3**) was concluded to be at the C-3 position from the low-field shift (δ 5.59) of 3-H.

Catalytic hydrogenation (Pd–C/EtOH) of (**2**) gave three products, among which one was readily identifiable with 2,3-di-*O*-galloyl-4,6-(*S*)-hexahydroxydiphenoyl-D-glucose.[7] The structure of the second one was established as 2,3-di-*O*-galloyl-4,6-(*S*)-dehydrohexahydroxydiphenoyl-D-glucose (**8**) by its tannase hydrolysis to yield a partial hydrolysate, which was identified as 4,6-(*S*)-dehydrohexahydroxydiphenoyl-D-glucose (**8**) derived from 1,2,3-tri-*O*-galloyl-4,6-(*S*)-dehydrohexahydroxydiphenoyl-β-D-glucose (trapain)[8] by similar tannase hydrolysis. The production of (**8**) clearly indicated that the absolute configuration of the methine carbon in a cyclohexenone ring located at the glucose C-6 position is in the *S*-series. The ^1H and ^{13}C NMR spectra [δ 1.8—2.1 (m), 29.0 (methylene), 3.69 (m), 72.3 (hydroxy-bearing methine)] and the negative FAB MS data [*m/z* 823 $(M–H)^-$] of the remaining product were consistent with the structure (**9**). The configuration of each carbon atom was established by examination of NOESY and ^1H–^1H COSY (Figure 1). Particularly, the *cis*-orientation of the two methine carbons (C-1 and C-1′) was further confirmed by NOE difference spectroscopy. Thus, the absolute configuration at the C-1′ position could be concluded to be in the *R*-series.

Carpinins A—D (**1**)—(**4**) represent the first examples of a new class of hydrolysable tannins in which two aromatic rings in the hexahydroxydiphenic acid ester group are dehydrogenated, and the characterisation of these compounds extends our knowledge of the metabolism of the widely found ellagitannins.

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