

Nuclear Deuterium Exchange in Methoxybenzenes and Methylated Flavonoids

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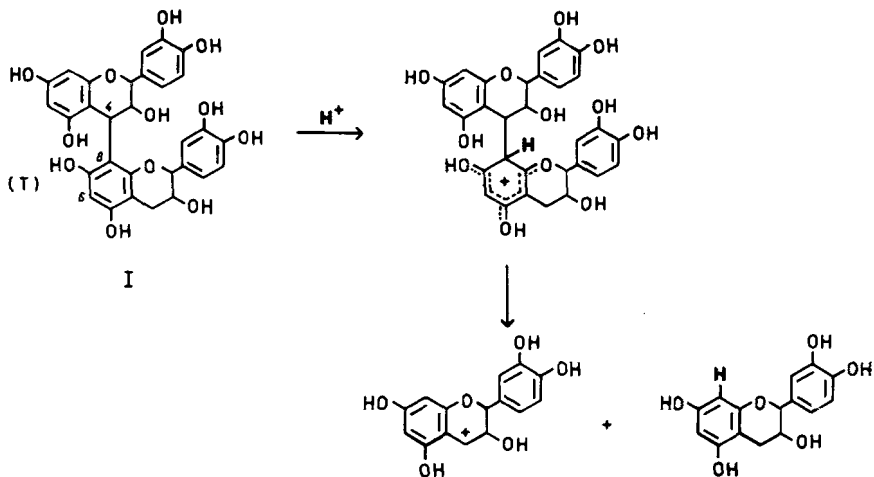
SUMMARY

The isolation of 6,8-d₂-catechin/epicatechin 5,7,3',4'-tetramethyl ether from the cleavage of a methylated condensed procyanidin with D₂O/dioxan resulted in an investigation of deuterium exchange in some related compounds under identical experimental conditions. In the absence of any acidic catalyst, a partial exchange was established in the majority of di- and trimethoxybenzenes when heated with D₂O/dioxan (3 : 1) at 95° for 16 hr in Pyrex glass ampules; 1,3,5-trimethoxybenzene was found to undergo a complete exchange of nuclear hydrogens under these mild conditions. Methylated flavonoids containing a reactive phloroglucinol system also exchanged both nuclear hydrogens at C₆ and C₈ with deuterium, but 5,7,3',4'-tetramethoxy-2,3-trans-flavan-3,4-cis-diol (2R : 3R-trans-R3 : 4S-cis) failed to react.

INTRODUCTION.

Condensed procyanidins of the general structure I are degraded into their constituent units by treatment with hot mineral acids, and the cleavage can be represented by the following mechanism ⁽¹⁾ :

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Although the structure of such a C_4 - C_8 linked dimer was confirmed by synthesis ⁽²⁾, the differentiation between substitution at C_6 or C_8 of the tail unit (T) is often difficult, and thus presents a major structural problem in many natural products. Since the acid catalysed cleavage can be carried out also with methylated compounds, it seemed advantageous to perform the reaction in DCl/D_2O to label specifically the aromatic terminus of the connecting bond. However, the catechin/epicatechin 5,7,3',4'-tetramethyl ether, obtained from the fission of a methylated procyanidin isolated from *Vitis vinifera* ⁽³⁾, was found to contain both nuclear hydrogens at C_6 and C_8 exchanged for deuterium. The same result was obtained when the cleavage was effected with 20% CH_3COOD in D_2O , or even in D_2O /dioxan mixture. This unexpected result prompted an investigation of the susceptibility of methoxybenzenes and methylated flavonoids to deuterium exchange under identical experimental conditions.

RESULTS AND DISCUSSION.

Nuclear deuterium exchange in methoxybenzenes and methylated flavonoids which were heated with D_2O /dioxan (3 : 1) at 95° for 16 hr in Pyrex glass ampules was determined by NMR spectroscopy from the decrease in the integrated areas of the ring hydrogens. The results are summarized in the Table below.

These results clearly show the accelerating influence of the number and positions of the substituent methoxyls on the rate of exchange under the mild experimental conditions reported. No detectable deuterium exchange in methoxybenzene and 1,2-dimethoxybenzene and the partial exchange established in 1,3-dimethoxybenzene, 1,2,3-trimethoxybenzene and 1,2,4-trimethoxy-

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	Exchange (%)		Exchange (%)
Methoxybenzene	0	1,2,3-Trimethoxybenzene	21.5
1,2-Dimethoxybenzene	0	1,2,4-Trimethoxybenzene	16.1
1,3-Dimethoxybenzene	36.2	1,3,5-Trimethoxybenzene	100
Catechin 5,7,3',4'-tetramethyl ether	100	Dihydroquercetin 5,7,3',4'-tetramethyl ether	0
5,7,3',4'-Tetramethoxyflavan	100	5,7,3',4'-Tetramethoxy-2,3-trans-flavan-3,4-cis-diol	0

benzene, seem to be in good agreement with the degree of activation of the ring by the electron releasing effect of the substituent methoxyls. However, the complete exchange of the nuclear hydrogens in 1,3,5-trimethoxybenzene, shown by the disappearance of the NMR signal (Fig. 1), is of particular interest.

Kresge and Chiang⁽⁴⁾ investigated the kinetics and mechanism of the acid catalysed aromatic hydrogen exchange in 1,3,5-trimethoxybenzene under a variety of conditions. The authors showed that the rate of exchange was proportional to the first power by hydronium ion concentration over the range 1.3×10^{-5} to 5×10^{-2} N, and conformed to the Brønsted relation $k_A = 4.72 \times 10^{-2} (K_A)^{0.518}$ in seven acids ranging in strength from H_3O^+ to H_2O . However, the reaction was not catalysed by base and no exchange was observed in sodium hydroxide solution during the time required for a complete exchange in acid of a comparable hydronium ion concentration. First-order kinetics which were observed indicated the absence of any chemical reaction other than hydrogen exchange, and this result was further supported by the constancy of UV absorption of the reaction mixtures, and by the high recoveries of the substrate. These facts were therefore inconsistent with the previously assigned mechanism for aromatic hydrogen exchange involving a reversible formation of a loose complex between the proton and aromatic substrate, followed by a slow intramolecular isomerization of the complex. Since the reaction rate was found to be proportional to the concentration of all acidic species present, it was concluded that the exchange was subject to general acid catalysis. Therefore, the mechanism had to be a consecutive slow proton transfer which takes place in two discrete steps : proton addition to give an intermediate phenonium ion, followed by proton abstraction to yield the exchanged trimethoxybenzene. This mechanism is also consistent with the mechanistic schemes accepted for other electrophilic aromatic substitutions.

It is therefore conceivable that in the absence of any acidic catalyst, the exchange was assisted by the Pyrex glass surface. Supporting evidence for this catalysis is the finding that both the cleavage of the methylated procyanidin and the exchange reactions invariably failed when attempted in soda glass NMR tubes.

Methylated flavonoids containing a reactive phloroglucinol system showed a similar susceptibility to deuterium exchange. A complete exchange of both hydrogens at C₆ and C₈ of ring A in catechin 5,7,3',4'-tetramethyl

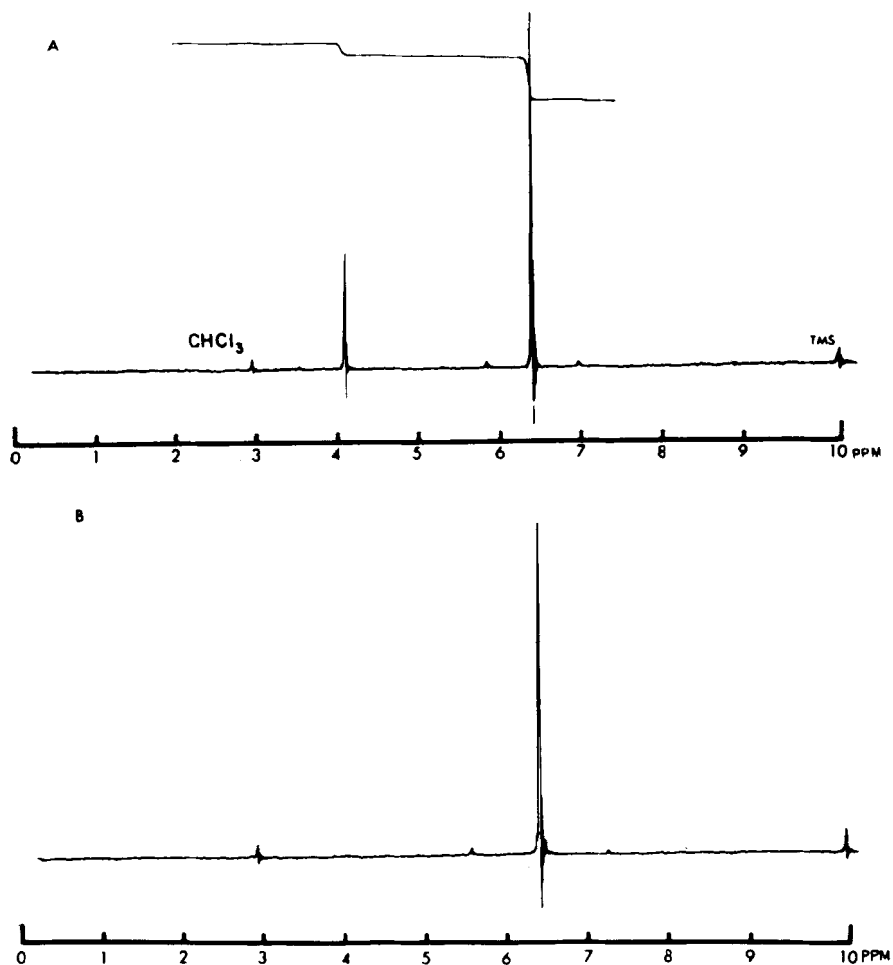


FIG. 1.

A. NMR spectrum of 1,3,5-trimethoxybenzene.

B. NMR spectrum of 1,3,5-trimethoxybenzene-2,4,6-d₃.

ether was established by NMR spectroscopy (Fig. 2) and confirmed by mass spectrometry. The molecular ion of the exchanged compound was found at m/e 348 ($C_{19}H_{20}O_6D_2$), and all ions derived from A ring showed an increase of two mass units in complete agreement with the structure of 6,8- d_2 -catechin 5,7,3',4'-tetramethyl ether.

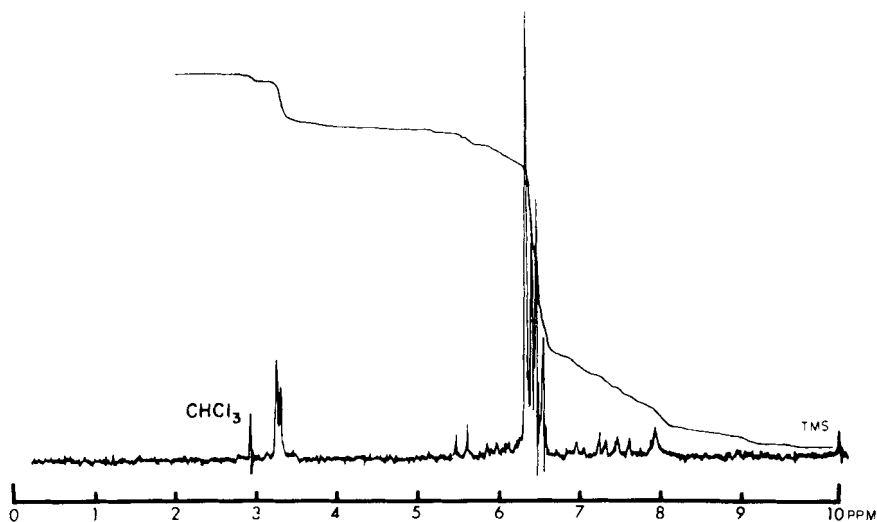


FIG. 2. NMR spectrum of 6,8- d_2 -catechin 5,7,3',4'-tetramethyl ether.

The same result was obtained with 5,7,3',4'-tetramethoxyflavan which also exchanged both hydrogens at C_6 and C_8 for deuterium. As expected, dihydroquercetin 5,7,3',4'-tetramethyl ether did not exchange, and this result can be satisfactorily explained by the deactivation of the A ring by the adjacent carbonyl group at C_4 . However, the inability of 5,7,3',4'-tetramethoxy-2,3-trans-flavan-3,4-cis-diol (2R : 3R-trans-3R : 4S-cis) to undergo deuterium exchange raised an interesting point : if the opening of the heterocyclic ring and hence the resulting carbonium ion were the determining factor, then the 4-hydroxyl could only be expected to enhance the stability by internal solvation. This does not seem to be the case. A possible explanation for this behaviour could be in the deprotonation of the intermediate phenonium ion by the lone pair of electrons of the adjacent 4-hydroxyl oxygen which can bend over the A ring to some extent. Oki and Iwamura⁽⁵⁾ showed by a comparative IR examination of the hydroxyl group absorption that benzyl alcohols and phenylethanols undergo such intramolecular protonation. Iwamura⁽⁶⁾ has later introduced a useful empirical correlation which allows the estimation of conformation in β -phenylethanols from the dihedral angle between the plane of the benzene ring and the bond between the carbon

atoms C_α and C_β of the side chain. Although not strictly analogous, an inspection of molecular models revealed that the largest value of the angle in the half-chair and half-boat conformations of 5,7,3',4'-tetramethoxy-2,3-trans-flavan-3,4-cis-diol was found to be 57° and 62° , respectively, and that a similar interference was possible.

EXPERIMENTAL.

General : NMR spectra were measured on a Perkin-Elmer R 10 spectrometer in $CDCl_3$ with tetramethylsilane as an internal standard, and mass spectra were recorded on an Atlas CH 4 instrument.

The methoxybenzenes were commercially available compounds.

Catechin 5,7,3',4'-tetramethyl ether and dihydroquercetin 5,7,3',4'-tetramethyl ether were prepared by methylation of natural products with dimethyl sulphate in acetone over anhydrous potassium carbonate ⁽⁷⁾.

5,7,3',4'-Tetramethoxy-2,3-trans-flavan-3,4-cis-diol was prepared by reduction of dihydroquercetin 5,7,3',4'-tetramethyl ether with potassium borohydride in methanol ⁽⁸⁾. The reduced product, consisting of a mixture of two epimers which were separated by fractional crystallization from methanol, yielded : 1. 5,7,3',4'-tetramethoxy-2,3-trans-flavan-3,4-trans-diol (2R : 3R-trans-3R : 4R-trans), compact crystals (28%), m.p. 202 (Lit. ⁽⁸⁾ m.p. 198); 2. the epimeric main product, 5,7,3',4'-tetramethoxy-2,3-trans-flavan-3,4-cis-diol (2R : 3R-trans-3R : 4S-cis), fine needles (64%), m.p. 172; the NMR coupling constants of the trimethylsilyl ether were $J_{2,3} = 9$ c.p.s., and $J_{3,4} = 2$ c.p.s.

5,7,3',4'-Tetramethoxyflavan, prepared by catalytic hydrogenation of 5,7,3',4'-tetramethoxyflavanone ⁽⁹⁾ over platinum oxide catalyst in dry dioxan, crystallized from ethanol in 72% yield and melted at 109 (Lit. ⁽¹⁰⁾ m.p. 111).

Exchange procedure : The compounds (0.1 g, 0.25-1.0 millimole) were dissolved in dioxan (15 ml) which was dried by a passage through a column of neutral alumina and the solutions, in thick-walled Pyrex glass ampules, were diluted with D_2O (45 ml, 99.8%). The sealed ampules were heated in a boiling water bath for 16 hr, and the exchanged compounds were isolated by ether extraction (3×30 ml). Since no decomposition was detected during the exchange, the residues were directly examined by NMR. To ensure that no back-exchange occurred during the work-up, any contact of the isolated compounds with acids, bases, or active surfaces was avoided.

The cleavage of the methylated procyanidin with 0.05 N HCl in D_2O , with 20% CH_3COOD in D_2O or with D_2O /dioxan mixture was carried out as described above. The reaction mixtures were extracted with ethyl acetate (3×30 ml) and the residues were separated by PLC ($R_F = 0.54-0.58$, benzene-acetone, 3 : 1); detection with vanillin in ethanolic HCl). After purification on a short column of neutral alumina (ether containing 1% ethanol) and crystallization from ethanol, the yield of 6,8- d_2 -catechin/epicatechin 5,7,3',4'-tetra-

methyl ether ranged from 24.4% for cleavage in D₂O/dioxan to 40.0% for cleavage in 20% CH₃COOD, expressed on the basis of one catechin yielding unit in the molecule.

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