PMR SPECTRA OF CATECHINS AND THEIR DERIVATIVES

Sh. Yu. Islambekov, A. K. Karimdzhanov,
A. I. Ismailov, F. G. Kamaev,
and A. S. Sadykov

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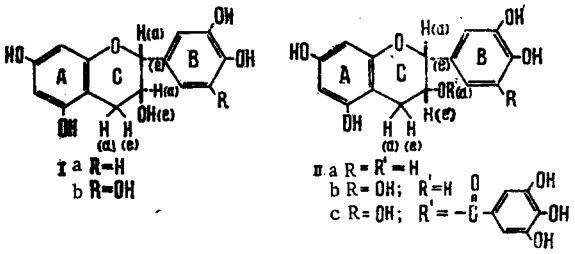
Proton magnetic resonance (PMR) spectroscopy is widely used in the investigation of the electronic and spatial structures of polyphenols. However, there are few publications devoted to the stereochemistry of the catechins, and the information given in them is contradictory [1-5]. In the present paper the structures of (+)-catechin, (±)-gallocatechin, (−)-epicatechin, (−)-epigallocatechin, and (−)-epigallocatechin gallate, isolated from Polygonum coriarium Grig. and Calligonum minimum Lipsky [6-8] and their acetyl derivatives, as studied with the aid of their PMR spectra, are considered.

In the PMR spectrum of (+)-catechin (Ia), the assignment of the signals of the aromatic protons of rings A and B (Fig. 1a) was made by analogy with the PMR spectra of the flavonoids [9-11].



A broad signal at 8.1 ppm that disappears when the sample is shaken with D_2O belongs to four hydroxyl protons of the phenol type, a signal at 6.86 ppm to H-2' and a signal at 6.72 ppm with an integral intensity corresponding to two protons to H-5' and H-6'. The H-8 and H-6 protons resonate at 5.98 and 5.84 ppm, respectively, in the form of doublets ($J_{6,8}$ = 2.4 Hz). The signals of the protons of the heterocyclic ring C are located in stronger fields. A doublet at 4.53 ppm relates to H-2, and a multiplet with a width of 21.7 Hz at 3.97 ppm to the H-3 proton. The $J_{2,3}$ spin−spin coupling constant is 7.9 Hz, which corresponds to the trans orientation of the H-2 and H-3 protons in ring C [4, 11]. The pseudoaxial orientation of these protons also follows from the values of the spin−spin coupling constants $J_{3,4e}$ = 5.6 Hz and $J_{3,4a}$ = 8.2 Hz. The H-4e and H-4a protons resonate in the form of quartets at 2.87 and 2.48 ppm respectively (J_{gem} = 16.5 Hz). Thus, H-2 and H-3 occupy pseudoaxial positions in ring C, which has the half-chair conformation, and the structure of (+)-catechin corresponds to that given in [4, 11], but not in [1].

An attempt has been made previously [11] to determine the conformation of ring C of catechins, but neither of the theoretical "half-chair" conformations agreed with the dihedral angles determined from the Karplus equation on the basis of the experimental values of the vicinal spin−spin coupling constants. This is apparently due to the fact that the observed constants are averaged values of the constants corresponding to several equilibrium forms.

The acetylation of catechin led to a downfield displacement of all the signals (Fig. 1b). The H-2', H-5', and H-6' aromatic protons of ring B are located in the 7.2-7.4 ppm region. The H-6 and H-8 atoms resonate at 6.55 and 6.64 ppm, respectively. A complex multiplet in the 5.15-5.35 ppm region is formed by the superposition of the signals of the H-2 and H-3 protons. The H-4e and H-4a protons also form second-order signals located at 2.60-2.90 ppm. In double-resonance experiments with the action of a strong high-frequency field on

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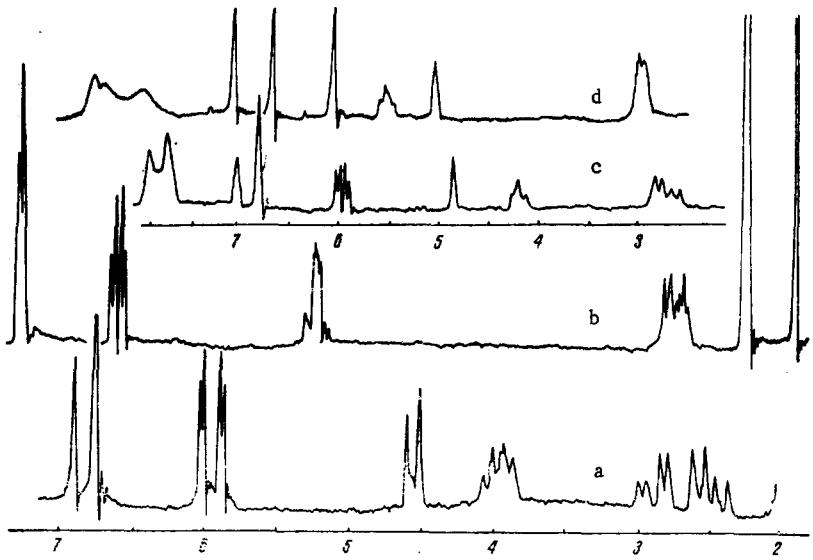

Fig. 1. PMR spectra: a) (+)-catechin (100 MHz); b) catechin pentaacetate (100 MHz); c) (−)-epicatechin (60 MHz); d) (−)-epigallocatechin gallate (60 MHz).

the signals of the C-4 protons, the H-2 and H-3 protons form an AB spin system $J_{2,3} = 8.1$ Hz, which shows the trans orientation of these protons. When a hydroxy group is acetylated, the signal of the geminal proton usually shifts downfield by 1.0-1.2 ppm [11, 12], as in the case for H-3. The substantial downfield shift for the H-2 signal (~ 0.7 ppm) is explained by the action of the equatorial 3-acetyl group, since it is just at this orientation that the neighboring H-2 axial protons (and also H-4) have the maximum downfield shift [12].

The signals of the four acetyl groups are located at 2.22 ppm, and the signal of the fifth is displaced upfield (δ 1.87 ppm). It may be assumed that this signal belongs to the acetyl group at C-3, since in the spectra of flavonol acetates [10] the acetyl groups of the sugar moiety resonate in a stronger field than those of the aromatic fragments. Furthermore, the anisotropy of ring C affects the shift of the signal of the C-3 acetyl group [10].

In the spectrum of (±)-gallocatechin (Ib) the presence of an additional hydroxy group in ring B leads to the equivalence of H-2' and H-6' signals (δ 6.43 ppm). The H-6 and H-8 signals are located at 5.83 and 5.97 ppm, respectively. The parameters of the H-2, H-3, and H-4 signals are identical with those of (+)-catechin, which shows the identical structures of ring C of these compounds.

The PMR spectrum of acetylated gallocatechin repeats the spectrum of (+)-catechin acetate in broad outlines, with the exception of the fact that the signal of one aromatic proton – H-5 – had disappeared and an additional acetyl group signal has appeared in the 2.2 ppm region.

Figure 1c shows the PMR spectrum of (−)-epicatechin (IIa). In the 7.5-8.1 ppm region there are the signals of four hydroxy groups of the phenol type. The signal at 7.0 ppm corresponds to H-2' and that at 6.76 ppm to H-5' and H-6' [9, 10]. The H-6 and H-8 signals resonate at 5.88 and 5.99 ppm, respectively. The H-2 signal in the form of a singlet is located at 4.85 ppm and has a width $\Delta W \approx 4$ Hz. Such a width of the signal shows the cis orientation of the H-2 and H-3 protons [11], since the vicinal constant is small. If the equatorial orientation of the aryl radical is assumed [4, 11], this is apparently connected with the pseudoaxial orientation of the hydroxy group in ring C of the epicatechin, which agrees with literature information [1, 4]. The H-3 proton resonates at 4.18 ppm in the form of a poorly resolved triplet having a width $\Delta W = 9$ Hz. The signals of the H-4a and H-4e protons form a second-order spectrum in the 2.7-2.9 ppm region.

The spectrum of (−)-epigallocatechin (IIb) contains a two-proton singlet at 6.53 ppm relating to H-2' and H-6', but it is otherwise identical with the spectrum of (−)-epicatechin and, consequently, the structure of (−)-epigallocatechin is analogous to that of (−)-epicatechin.

In the spectrum of (−)-epigallocatechin gallate (IIc) (Fig. 1d), the signals of the hydroxy groups are located in the 7.8-8.5 ppm region. In 6.0-7.0 ppm region there are three singlets which may be assigned in the following way on the basis of a comparison with the compounds already considered. The singlet at 5.98 ppm is

due to the H-6 and H-8 protons, the singlet at 6.57 ppm to the H-2' and H-6' protons, and the singlet at 6.98 ppm to the H-2" and H-6" aromatic protons of the additional ring. The distribution of the H-2 and H-3 signals in the spectrum of (−)-epigallocatechin gallate is different from that in epigallocatechin. The broad signal of the H-3 proton (ΔW = 9 Hz) is located in a weaker field (5.47 ppm) than the comparatively narrow H-2 signal (5.0 ppm). This is due to the influence of the carbonyl-containing substituent located just in the C-3 position; a similar effect is observed in the PMR spectrum of the acetyl derivative of (−)-epigallocatechin, in which the H-3 signal is found at 5.26 ppm and the H-2 signal at 4.97 ppm.

Thus, when the OH group at C-3 of epigallocatechin is acetylated, the H-3 signal shifts downfield by 1.3 ppm, as has been observed in the case of catechin, but in contrast to the latter the chemical shift of the H-2 proton changes inconsiderably, which is due to the axial orientation of the substituent.

The remaining signals of (−)-epigallocatechin acetate are arranged in the following way: The equivalent protons H-2' and H-6' resonate at 7.03 ppm and the H-6 and H-8 protons at 6.46 and 6.57 ppm, respectively. The signals of the two protons at C-4 are located in the 2.84-ppm region. The protons of the five acetyl groups give signals at 2.23 ppm and the signal of one acetyl group is located at 1.86 ppm.

The spectrum of (−)-epigallocatechin gallate contains the following signals: H-2' and H-6' at 7.36 ppm; H-2" and H-6" at 7.17 ppm; H-8 at 6.64 ppm; H-6 at 6.53 ppm; H-3 at 5.58 ppm; H-2 at 5.1 ppm; and H-4 (2) at 2.98 ppm. The signals of the eight acetyl groups are located at 2.20 ppm, and in this spectrum there is no signal of an acetyl group at 1.8-1.9 ppm such as has been observed in the spectra of all the acetyl derivatives of catechins considered previously. This confirms the hypothesis that the signals of an acetyl group at C-3 are located in the 1.8-1.9 ppm region.

A comparison of the PMR spectra of the catechins and their acetates shows that in the latter the signals of all the aromatic protons are shifted downfield in comparison with the corresponding signals in the spectra of the initial compounds.

EXPERIMENTAL

Recording of the PMR Spectra. The samples used for recording the PMR spectra were 8-10% solutions of the substances under investigation in deuteroacetone and deuterochloroform. The chemical shifts are given in the δ scale relative to the signal of HMDS as internal standard. The spectra were recorded on Hitachi H-60 and Varian XL-100 spectrometers.

Isolation of the Catechins. The chromatographic separation and isolation of (+)-catechin, (−)-epicatechin, (−)-epigallocatechin gallate, (±)-gallocatechin, and (−)-epigallocatechin, and their properties, have been reported previously [6, 7].

Acetylation of the Catechins. A catechin (0.1 g) was dissolved in 3 ml of pyridine. With constant stirring, 5 ml of acetic anhydride was added to the solution. The mixture obtained was left for 24 h. Then it was poured into ice water with stirring and after 2-3 h the precipitate was filtered off and dried in a vacuum desiccator over P_2O_5. The acetyl derivatives obtained were recrystallized from acetone−petroleum ether (1 : 5). The elementary analysis corresponded to the calculated figures.

SUMMARY

The PMR spectra of catechins, gallocatechins, epigallocatechin gallate, and their acetyl derivatives have been analyzed and the structures of the compounds investigated have been confirmed.

The parameters of the signals of the H-2 and H-3 protons that have been found and also the nature of their change on acetylation enable a compound investigated to be assigned unambiguously to the normal or to the epi series.

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ESSENTIAL OILS OF MONGOLIAN PLANTS

A STUDY OF THE ESSENTIAL OIL OF Artemisia rutifolia

A. L. Shavarda

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The aim of the present work was to study the essential oil of Artemisia rutifolia Steph. ex Spreng. The material was collected by the Division for the Study of Resources of the Combined Soviet–Mongolian Comprehensive Biological Expedition in 1972 in the budding–flowering phase in the Ubsunur Iamak, close to lake Dzérén-Nur in sections of a pebbly-sandy desert. The essential oil of Artemisia rutifolia growing in the Pamir was investigated by M. I. Goryaev in 1959 [1].

According to preliminary results which we obtained by means of an analytical gas chromatograph, the composition of the essential oil of the Artemisia rutifolia growing in Mongolia is far more complex (Fig. 1). By preparative gas chromatography in column I (Table 1), we obtained a fraction of the readily volatile components present in the oil in trace amounts. Its composition was studied by analytical gas chromatography on column V (see Table 1), identification being made by the addition of authentic compounds. The chromatogram of the monoterpine fraction is shown in Fig. 2. Six compounds were identified: α-pinene, camphene, β-pinene, limonene, β-phellandrene, and p-cymene.

The oxygen-containing compounds were isolated by preparative gas chromatography using column I and were identified by physical and chemical methods [2, 3]; the purity of the compounds isolated was checked by analytical gas chromatography in columns II, III, and IV (see Table 1).

The complete composition of the oil is shown in Table 2. The compounds corresponding to peaks 11-16 could not be identified from their IR spectra.

The IR spectrum of substance (11) showed the absorption bands of a monosubstituted aromatic ring (700, 760, 1500 cm^{-1}) and of a carbonyl group (1715 cm^{-1}). According to its mass spectrum (M^+ 148), the substance had the formula $C_{10}H_{12}O$. In its NMR spectrum, in addition to the protons of the aromatic ring and of an acyl group, there is a multiplet (2.80 ppm, 4 H) of the protons of two neighboring methylene groups. On the basis of this spectral information, substance (11) was identified as 4-phenylbutan-2-one. The IR spectrum of 4-phenylbutan-2-one which we obtained by the hydrogenation of the product of the condensation of benzaldehyde with acetone proved to be completely identical with the IR spectrum of the natural compound (11).

Compound (13) is an aromatic alcohol. Its IR spectrum contains absorption bands of a monosubstituted aromatic ring (700, 750, 1500 cm^{-1}) and of a hydroxy group (1060, 1130, 3390 cm^{-1}). The NMR spectrum has the signals of aromatic protons and of a $CH_3-CH(OH)$ grouping (see Experimental). In addition there are two multiplet signals corresponding to two methylene groups. The mass spectrum has a strong M^+-18 peak (70%),

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