New A-type Trimeric and Tetrameric Procyanidins from Peanut Skins

Marta K. Jamróz¹, Matthew H. Davey², Sławomir Kaźmierski³, Witold Danikiewicz⁴, Grzegorz Spólnik⁴, Jan A. Gliński²

¹Department of Physical Chemistry, Faculty of Pharmacy, Medical University of Warsaw, 1 Banacha Street, 02-097 Warsaw, Poland, ²Planta Analytica, LLC, 39 Rose Street, Danbury, CT 06810, USA, ³The Centre of Molecular and Macromolecular Studies PAS, Sienkiewicza 112, Lodz, Poland, ⁴Organic Chemistry Institute PAS, Kasprzaka 44/52, 01–224 Warsaw, Poland

ABSTRACT: Peanut skins constitute up to 4% w/w of peanuts and are a unique source of A-type procyanidins. Methanol extract of the skins was purified by Centrifugal Partition Chromatography (FCPC Kromaton, Roussel Robatell; France) and RP HPLC to afford pure procyanidins. They contain both epicatechin (EC) and catechin (C) subunits, which are joined through 4β–8 or 4α–6 bonds. The extract contains dimers A₁ and A₂ and a mixture of tetramers, monomers, and trimers. Two new tetrameric procyanidins containing two A–type bonds and one trimer, in addition to three known trimers, were isolated. The structures were established to be EC–(4β–8), 2β–O–7'–EC–(4β–8')–EC–(4β–8)–2β–O–7'–C (1), EC–(4β–8), 2β–O–7'–EC–(4β–8')–EC–(4β–8)–2β–O–7'–C (2), EC–(4β–8), 2β–O–7'–EC–(4β–8')–EC–(4β–8)–2β–O–7'–C (3), EC–(4β–8), 2β–O–7'–EC–(4β–8')–EC–(4β–8)–2β–O–7'–C (4), EC–(4β–8)–EC–(4β–8')–EC–(4β–8)–2β–O–7'–C (5), EC–(4β–8)–EC–(4β–8')–EC–(4β–8)–2β–O–7'–C (6) by means of NMR and MS. Compound (4) was previously isolated from Litchi chinensis [1], compounds (5) and (6) were previously isolated from Vaccinium vitis-idaea [2].

Fig 1. Structure of two new tetramers from peanut skins.

Fig 2. Structure of four trimers isolated from peanut skins.

BACKGROUND AND RESULTS: Peanut skins are a byproduct of the peanut industry, which is rich in procyanidins. The compounds are believed to have protective effect for the cardiovascular system, however, due to scant supply their biological properties remain largely undetermined. In addition, there is still a lack of detailed data on their structures. The NMR data exist only of two easily distinguishable rotamers at 275K, whereas at 295K, the temperature at which most NMR measurements are conducted, the proton signals are broad and lose their multiplet structure. Interestingly, for one trimer and one tetramer with the A-linkage [3]. Recently, studies with MS spectroscopy showed a great variety of compounds (1 and 2, Fig. 1) are new compounds, whereas the trimers (3 – 6, Fig. 2) have been known, but originated from the different sources. A major obstacle to the structure determination of procyanidins is often caused by the fact that one molecule can exist in the form of different rotamers, which produce separate NMR signals. In this work rotamers of (6), one of the main peanut skin procyanidins trimers were studied in detail. The ¹H NMR spectra for cpd. 6 in the temperature range of 260K – 330K (Fig. 3) show the presence of two easily distinguishable rotamers at 275K, whereas at 295K, the temperature at which most NMR measurements are performed, the proton signals are broad and lose their multiplet structure. Interestingly, for (5), which differ from (6) only by 4-8 linkage (instead of 4-6) between top and middle unit, the multiplet structure in the ¹H NMR spectrum is preserved at 295K, suggesting higher rotational barrier for 4-8 linkage. The rotamers are observable for (3), but one of the rotamers is evidently energetically more favored than the other, since their mutual ratio is equal to 3:5:1. The optimized structure of (6) (DFT/B3LYP/6-31+G*) was partially re-optimized varying the C10-C4-C6'-C5' torsion angle value by 30°. Figure 4 shows the energy dependence of (6) on the C10-C4-C6'-C5' torsion angle. The two minima for 120° and -30° indicate the preferred conformations of the rotamers. The NOESY spectra for (6) at 275K with different mixing time (Fig. 5) were registered, showing the NOESY correlations within one of the rotamers when mixing time did not exceed 50 ms and correlations between different rotamers with mixing time equal to 100 ms and higher. Thus, one can estimate the lifetime of the rotamers.

Materials and Methods: Peanut skins were kindly donated by Universal Blanchers, Sylvester, GA. After defatting with hexane, they were extracted first with MeOH and then with 70% acetone. Both extracts were combined. Initial separation was done on silica gel. The fractions were subsequently subjected to further purification by Centrifugal Partition Chromatography with a L rotor (FCPC Kromaton, Roussel Robatell; France). Final purification was conducted on a YMC ODS-A column 150 x 30 mm 5 micron afford pure procyanidins. The HPLC analysis was conducted on an Hewlett Packard HPLC Model 1050 consisting of an autosampler, quaternary pump and DAD. A YMC column, PackPro C18, 150 x 4.6 mm, 3 micron was used (Fig.7). The ¹H NMR spectra were recorded at 500.12 MHz on a Bruker DSX-500 instrument. Standard pulse programs from Bruker library were used fro DEPT, COSY, HMQc, ROESY, and HMBC experiments.

Fig 3. The ¹H NMR spectra for (6) in the temperature range of 265K – 325K

Fig 4. The energy dependence on the C10-C4-C6'-C5' torsion angle for (6)

Fig 5. The NOESY spectra for cpd. 6 at 275K with different mixing time (50 ms – 500 ms)