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UV Spectrometric and DC Polarographic Studies on Apigenin and Luteolin

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Remarks on polyphenolic compounds has been arisen since past few years. The flavonoids appears to be the important groups of compounds with their capability to inhibit DNA damage, lipid peroxidation, to quench free radicals and, at least, anticarcinogenic and antiproliferative effects. On the other hand, their mechanism of action is still unexplained. Apigenin and luteolin are the most wide-spread flavones and they exhibited to be useful in chemoprevention. UV spectrometric and DC polarographic studies on these two compounds have been carried out with regard to changing pH. The most significant changes were observed at basic pH. These results could aid to elucidation of their mechanism of action as pH is one of the important factors for bioprocesses passing in living organisms.

Key words : Apigenin, Luteolin, UV spectrometry, DC polarography

INTRODUCTION

Flavonoids are naturally occuring polyphenolic compounds that are ubiquitous in plant food and important components of the human diet (Cai *et al.*, 1997) Over 4000 different flavonoids have been described and they are categorized into flavonols, flavones, flavanones, catechins, anthocyanidines and isoflavonoids (Hollman and Katan, 1997).

Rusznyak and Szent-Györgyi observed in 1936 that the mixture of two flavanones decreased capillary permeability and fragility in humans (Rusznyak and Szent-Györgyi, 1936). Recently, in amount of studies were exhibited their numerous biological capabilities, e.g. to act as anti-allergenes (Middleton and Drzewiecki, 1984; Cheong *et al.*, 1998), to inhibit DNA damage (Halliwell, 1996; Noroozi *et al.*, 1998), to inhibit lipid peroxidation and to quench free radicals (Fraga *et al.*, 1987; Candlish and Das, 1996). Lipid peroxidation should be correlated with other effects, e.g. with radical scavenging potential (Bors *et al.*, 1997; Otero *et al.*, 1997), cytotoxicity (Ramanathan *et al.*, 1994), metal chelation (Ratty, 1988; Moran *et al.*, 1997) or inhibitory effects on specific enzymes (Cos *et al.*, 1998; Ursini *et al.*, 1994). The results of the structure-activity relationship studies are basically consistent with the following structural criteria for optimal inhibition of lipid peroxidation: a catechol group in the B-ring, 2,3-double bond conjugated with the 4-oxo function and a 3- (and 5-) hydroxy group (Bors *et al.*, 1997; Uda *et al.*, 1997). At least, the interest in flavonoids has arisen because of their potential role in prevention of human cancer (Mitscher *et al.*, 1996; Cai *et al.*, 1997). The mechanisms responsible for their anticarcinogenic and antiproliferative effects are not clear but appear to be related to their antioxidant effect (Mora *et al.*, 1990; Morel *et al.*, 1993), free radical potential (Robak and Gryglewski, 1988), radioprotective effect (Shimoi *et al.*, 1994) and induction of apoptosis (Wei *et al.*, 1994; Plaumann *et al.*, 1996). On the other hand, most of studies show that they can also act as prooxidants under certain conditions (Aruoma *et al.*, 1992; Moran *et al.*, 1997).

The flavones, apigenin (AP) and luteolin (LU), belong to the most wide-spread flavones and they exhibited to be useful in chemoprevention (Steinmetz and Potter, 1996; Potter and Steinmetz, 1996). The UV spectrometric and DC polarographic studies were performed with AP and LU. The UV spectra and polarographic waves were recorded at various pH values. It is known that pH initiates the change of chromophoric groups. The change of pH in biological system can change the bioactivity of compound. As the benefit of dietary phenolics to health is strong, however, their mechanism of action is still unexplained, the focus on our study is to contribute to this elucidation.

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MATERIALS AND METHODS

Chemicals

LU and AP were purchased from Sigma-Aldrich Chemie (Steinheim, Germany). Methanol was purchased from Merck (Darmstadt, Germany). All compounds for preparation of Britton-Robinson buffer (BR buffer) were of analytical grade and were obtained from Fluka (Buchs, Switzerland).

UV spectrometry

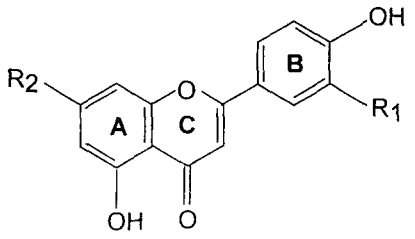
UV spectra of LU and AP were recorded with a Perkin Elmer 557 double beam wavelength spectrometer in the wavelength range 190~500 nm. The studied compounds were dissolved in methanol and then diluted in methanol, water or in BR buffer (the ratio of methanol to water or BR buffer was 1:9 in final solution) to final concentration of 5×10^{-5} M LU resp. 1×10^{-4} M AP. pH was controlled by pH-meter Radelkis (Budapest, Hungary) and in the case of need adjusted with a few drops of 0.2 M NaOH.

DC polarography

The method for the polarographic determination has been described earlier (Novotny and Vachalkova, 1993). All polarograms were recorded using a PA 4 polarographic analyzer equipped with a model 4106 two-line recorder (Laboratorni pristroje, Prague, Czech Republic). The concentration of compounds used was 10^{-4} M. They were directly diluted in polarographic cell in BR buffer.

RESULTS AND DISCUSSION

UV spectrometry has become a major technique for the structure analysis of flavonoids because of these two reasons. The first is that only a small amount of compound is required. The second is that the amount of structural information gained from UV spectrum can be enhanced by the use of specific reagents which react with one or more functional groups on the flavonoid skeleton (Harborne *et al.*, 1975).



	R_1	R_2
Apigenin	H	OH
Luteolin	OH	OH

Fig. 1. Chemical structure of flavones

The UV spectra of most flavonoids consists of two major absorption maxima, one of which occurs in the range 240~285 nm (band II belonging to A-ring benzoyl system) and the other in the range 300~400 nm (band I belonging to B-ring cinnamoyl system) (Fig. 1) (Harborne *et al.*, 1975; Dinya *et al.*, 1986). The absorption of flavones is markedly affected by the presence of OH-groups on A- resp. B-ring. Highly hydroxylated flavones tend to absorb at longer wavelengths. This knowledge is associated with the presence of OH-groups above all on B-ring. The absorption of A-ring demonstrated in UV spectrum as band II is less dependent on hydroxylation of ring (Harborne *et al.*, 1975).

The UV spectra of LU and AP were recorded in methanol, water (+10% methanol) and in BR buffer (+10% methanol) at various pH values.

The absorption maxima of LU were consistent with

Table I. Absorption maxima of LU and AP depending on pH

	Luteolin	Apigenin
pH	λ_{max}, λ_{sh} [nm]	
2,35	250 sh, 261, 286 sh, 342	264, 286 sh, 336
2,87	220 sh, 253 sh, 264, 287 sh, 348	264, 286 sh,335
4,10	254 sh, 262, 287 sh, 348	219 sh, 265, 285 sh, 336
5,02	220 sh, 253 sh, 260, 285 sh, 348	234 sh, 265, 286 sh, 337
6,05	225 sh, 257, 290 sh, 359	233 sh, 265, 338
7,00	224 sh, 255, 293 sh, 368	230, 266, 295 sh, 350
7,54	228 sh, 259, 298 sh, 372	226 sh, 271, 296 sh, 372
7,97	228 sh, 260, 320 sh, 375	226 sh, 271, 296 sh, 372
9,15	229 sh, 260, 270 sh, 322 sh, 378	226 sh, 274, 319 sh, 390
10,88	230 sh, 262, 325 sh, 384	225 sh, 272, 319 sh, 389
methanol	242sh, 254, 267, 291 sh, 352	268, 298 sh, 338
methanol/water (1:9)	252 sh, 264, 290 sh, 345	266, 340

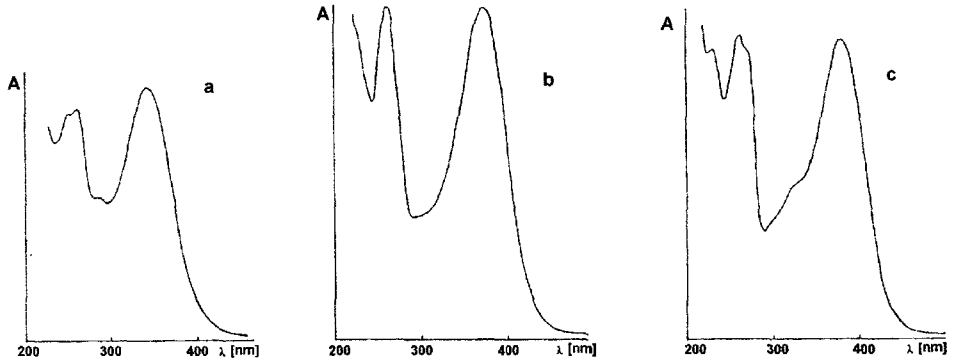


Fig. 2. UV spectrum of luteolin in Britton-Robinson buffer:methanol (9:1) at pH a) 2.35; b) 7.00; c) 9.15

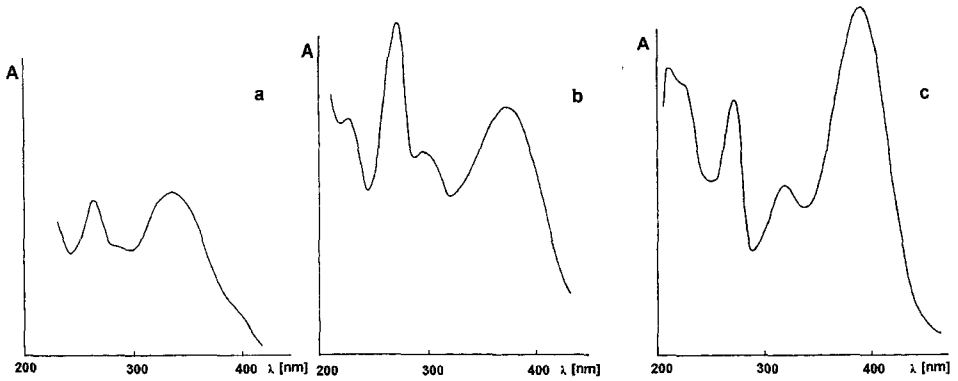


Fig. 3. UV spectrum of apigenin in Britton-Robinson buffer:methanol (9:1) at pH a) 2.35; b) 7.96; c) 10.88

the data published by Harborne *et al.* (Harborne *et al.*, 1975). The effect of water probably resulted in the lost of shoulder at 242 nm as well as in the shift of absorption bands I and II to shorter wavelengths. The absorption maxima and shoulders of LU at various pH ware in the Table I and UV spectra ware depicted in the Fig. 2. At pH 2.87, the shoulder was observed at wavelength 220 nm with low intensity. This shoulder became more pronounced at more basic pH and was shifted to longer wavelengths. The change of pH from 2.35 to 4.10 resulted in the decline of absorption intensity of all peaks in the spectrum. On the other hand, at pH 5.02 a mild increase in the absorption intensity was observed which was rapidly enhanced at pH 6.05 and 7.00. At higher pH, no change in intensity was observed. The solutions of LU with the pH above 6.05 were light-yellow coloured. It is evident (see Table I), that the shift of pH to higher values leads to the shift of band II (benzoyl chromophore) to shorter wavelength (hypsochromic effect) and to the shift of band I (cinnamoyl chromophore) to longer wavelength (bathochromic effect). The shoulder to the band II was lost at basic pH and the shoulder to the band I was shifted to longer wavelength.

The results of experiment with AP were also summarized in the Table I and were shown in Fig. 3. The shoulder to band I at 298 nm in UV spectrum of AP measured in methanolic solution was lost in water solution. This shoulder appeared again in buffer solution. The wavelength of bands I and II became longer with the shift of pH to higher values. Bathochromic effect was pronounced at band I and it was also accompanied with the change of colour of solution (deep yellow in basic solution). At pH 4.10, the shoulder was appeared at wavelength 219 nm which was mildly shifted to longer wavelength. The similar effect was observed at shoulder to band I. Its intensity was very low at pH from 2.35 to pH 5.02, at pH 6.05 the shoulder disappeared and the intensity of the shoulder significantly increased at alkaline pH (hyperchromic effect).

As the available data showed, the polarographic study of AP was described only by a few of authors (Brezina and Zuman, 1952). LU was not polarographically studied yet. Respecting the given information, the polarographic behaviour of LU and AP under various pH values has been investigated. AP was polarographically reduced on a dropping mercury electrode in one resp. two steps. At acidic pH (2.87~5.02), the reduction of AP proceeded in one two-electron step (Fig. 4, curve a). The reduction process changed when pH was shifted to neutral value. The original polarographic wave became separated to two new independent waves at pH 6.05. The change in polarographic behaviour at pH of 6.05 also demonstrated in UV spectrum disappearing the shoulder at 286 nm. The

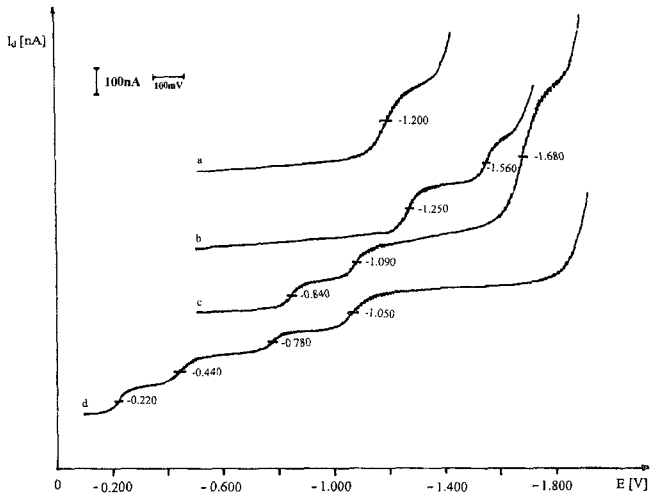


Fig. 4. Polarographic reduction of apigenin in Britton-Robinson buffer at different pH. Curves: (a) 2.87; (b) 6.05; (c) 9.15; (d) 10.88. Scan rate 5 $mV.s^{-1}$

half-wave potential $E_{1/2}$ of I. polarographic wave became more positive and the II. polarographic wave was more negative (Table II) when pH was changing to neutral value. Similarly as at spectrometric measurements, the colour of solution has changed (from colourless to yellow). At pH of 9.15, the new polarographic wave was recorded at $E_{1/2}$ -0.840 V. This wave, as well as the second wave ($E_{1/2}$=-1.090 V), were shifted to positive $E_{1/2}$ (-0.780 V) at strong alkaline pH 10.88. The third wave with $E_{1/2}$ of -1.680 V at pH of 10.88 disappeared and yellow colouring of solution became more deeper. Respecting the change described, the pronounced shift of λ_{sh} and λ_{max} I, II was observed in UV spectrum of AP at pH of 9.15.

Table 2. Half-wave potentials ($E_{1/2}$) of apigenin and luteolin depending on pH

pH	Apigenin $E_{1/2}$ [V]	Luteolin $E_{1/2}$ [V]
2.35	-1.200	-0.360
2.87	-1.200	-0.370
4.10	-1.300	-1.260
5.02	-1.340	-1.315
6.05	-1.250	-1.290
	-1.560	-1.540
7.00	-1.210	-1.155
	-1.610	-1.610
7.54	-1.200	-1.050
	-1.580	-1.650
7.97	-1.150	-1.000
	-1.600	-1.700
9.15	-0.840	-1.750
	-1.090	
	-1.680	
10.88	-0.220	-0.700
	-0.440	
	-0.780	
	-1.050	

LU compared to AP was better soluble at acidic pH. LU was reduced in one two-electron diffuse and reversible step under strong acidic condition (pH of 2.35 and 2.87). At pH of 4.10, the original wave of LU waned and new well defined wave at $E_{1/2}$ of -1.260 V vs. SCE was observed (Fig. 5, curve b). The $E_{1/2}$ of new wave became more negative with increasing of pH. At pH of 6.05, this wave was divided into two individual waves (Fig. 5, curve c). The change in reduction process was accompanied with the change of solution colouring (from colourless to intensive light-yellow under alkaline condition). The similar shift of polarographic waves occurred at neutral pH as in the case of AP. The polarographic reduction of LU waged at basic pH in one very negative step which faded out at pH of 10.88 (see Table II).

LU (3',4',5,7-tetrahydroxyflavone) and AP (4',5,7-trihydroxyflavone) are structurally different from one another with one more OH-group on B-ring of LU. In general, LU appears to be more symmetric molecule than AP. UV spectrum of the both flavones as well as their polarographic behaviour in BR buffer depends on pH. The shift of λ_{sh} and λ_{max} I to longer wavelength and enhancing of peak intensity were recorded at the change of pH from acidic to neutral. The new negative wave has arisen under alkaline conditions and its $E_{1/2}$ values became more positive. The results showed that the values of λ_{sh}, λ_{max} and $E_{1/2}$ were markedly changed under strong basic conditions.

It is evident that the OH-groups play important role and their localization on the nucleus is also not negligible. Four OH-groups of LU occurring on the ring affected the increase in peak intensity and bathochromic effect (Harborne *et al.*, 1975). These findings was observed more intensively in UV spectrum of AP, however, these changes would be associated with the

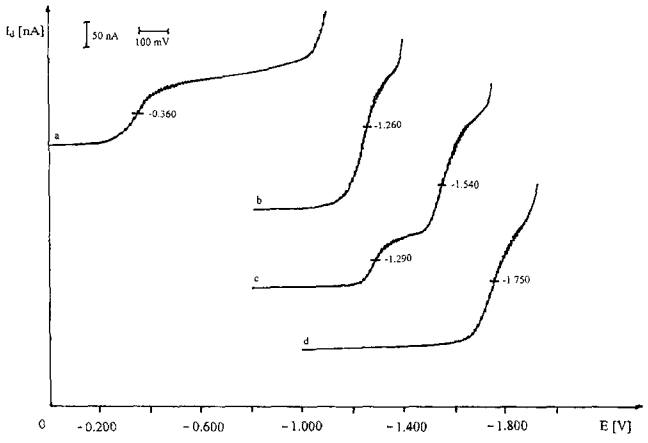


Fig. 5. Polarographic reduction of luteolin in Britton-Robinson buffer at different pH. (a) 2.35; (b) 4.10; (c) 6.05; (d) 9.15. Scan rate 5 $mV.s^{-1}$

change of original structure with its free electron pairs. The electron transfer on benzoyl system could be responsible for the origin of the shoulder to band II. The greater changes observed in UV spectrum and in polarogram of AP could be also due to unequal distribution of electron density in the consequence of presence of OH-groups on B-ring.

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