SYNTHESIS AND CHARACTERIZATION OF *N*-[2-(4-METHOXYPHENYL)ETHYL]OLEAMIDE

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This work is devoted to the synthesis and electrochemical characterization of N-[2-(4-methoxyphenyl)ethyl]oleamide. Its synthesis was realized by esterification of oleic acid to methyl oleate and reaction of this ester with N-2-(4-methoxyphenyl)ethylamine in anhydrous methanol, catalized by sodium methoxide. The new oleamide was fully characterized by IR, MS, ^{1}H- and ^{13}C-NMR spectra. Electrochemical investigations were also performed by cyclic and differential pulse voltammetry.

Keywords: *N*-[2-(4-methoxyphenyl)ethyl]oleamide, IR, MS, ^{1}H- and ^{13}C-NMR spectra, cyclic voltammetry, differential pulse voltammetry

1. Introduction

It is already known that *N*-acylethanolamides (NAEs) are a group of lipid mediators synthesized in the organism as a response of a many physiological and pathological stimuli, like obesity, inflammation and food intake. Between them, *N*-oleoylamides and also *N*-arachinoylethanolamides were investigated very intensively, especially in appetite and obesity regulation [1,2]. Similarly to oleylamides, *N*-palmitoylethanolamide (PEA) [3] and *N*-stearoylethanolamide (SEA) [4] were studied in relation to antiinflammatory and apoptotic properties. In the same time, from the group of macamides [5] (benzamide derivatives of saturated and unsaturated C-18 fatty acids), discovered in the Maca (Lepidium meyenii) plant, a traditional food crop of the Peruvian Andes now widely touted

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as a dietary supplement, five of them are oleylbenzylamides substituted in *meta*, or pyridylmethyl-6 substituted amides.

Due to their importance, our group was involved in the synthesis of new oleamides in order to find useful properties by introducing new fragments in the amide part of the molecule. The present paper concerns the synthesis of *N*-[2-(4-methoxyphenyl)ethyl]oleamide **1** (Fig. 1) which was characterized physico-chemical and electrochemical methods prior to determination of its biological activity.

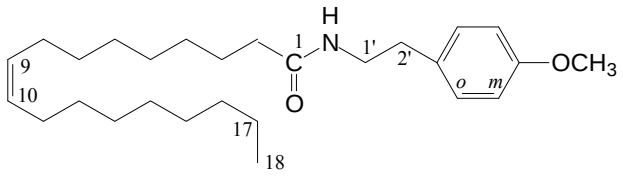

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Fig. 1. Structure of *N*-[2-(4-methoxyphenyl)ethyl]oleamide

2. Experimental

2.1. Reagents and procedure

All compounds used for synthesis were purchased from Merck and used without further purification. As solvent and supporting electrolyte acetonitrile and tetrabutylammonium perchlorate from Fluka were used (as received).

Melting point was determined with OptiMelt. The ^{1}H- and ^{13}C-NMR spectra were recorded using Varian Gemini 300 BB instrument, operating at 300 MHz for ^{1}H- NMR and at 75 MHz for ^{13}C-NMR, with $CDCl_3$ as solvent and TMS as internal standard. The IR spectra (ATR) were recorded on a Vertex 70 Brucker instrument. Mass spectra were recorded using LTQ Orbitrap Velos Pro, by injecting a solution of 100 fmol/µl in 0.1 % formic acid in methanol. The spectrum was acquired in SIM scan mode (416.35 ± 10 Da).

The electrochemical experiments were carried out using a PGSTAT12 AUTOLAB potentiostat coupled to a three-compartment cell. The CV curves were generally recorded at 0.1V/s or at various rates (0.1 - 1V/s) when studying the influence of the scan rate. DPV curves were recorded at 0.01V/s with a pulse height of 0.025V and a step time of 0.2 s. The working electrode was a glassy carbon disk (diameter of 3mm). The active surface was polished before each determination with diamond paste (200 µm). The Ag/10 mM $AgNO_3$ in 0.1 M TBAP, CH_3CN was used as reference electrode. The potential was referred to the potential of the ferrocene/ferricinium redox couple (Fc/Fc^{+}) which in our experimental conditions was +0.07V. A platinum wire was used as auxiliary electrode. The electrochemical experiments were performed at 25ºC under argon atmosphere.

2.2. Synthesis of compound 1

a. Synthesis of methyl oleate

Methyl oleate was prepared by esterification of oleic acid with methanol catalyzed by p-toluenesulfonic acid (Method A) and also by a selective method (B) which does not affect the double bond of the molecule in anhydrous mixture of THF-HMPT, when the acid is transformed in its potassium salt with $KHCO_3$, which is then esterified with an excess of methyl iodide.

Method A

28.2 g (0.1M) oleic acid in 150 mL anhydrous methanol and 1g p-toluenesulfonic acid were refluxed for 6 h, monitoring the reaction by thin layer chromatography (TLC) on Merck silica gel plates with ethyl acetate-methanol, 90:13, as eluent system; $R_{f\ oleic\ acid} = 0.60$; $R_{f\ methyl\ oleate} = 0.80$. After usual work-up, 27.5 g (yield 95%) crude methyl oleate was obtained.

Method B

2.82g (10mmoles) oleic acid were dissolved in 20 mL tetrahydrofuran (THF) and 10 mL hexamethylphosphoroustriamide (HMPT), 1.4g (14 mmoles) $KHCO_3$ were added; the mixture was stirred for 30 minutes, then 50 mL methyl iodide were added and the mixture was stirred again in the same vessel for 24 hours, monitoring the reaction by TLC as in the method A. THF was removed by distillation under vacuum, 50 mL water were added and the product was extracted with benzene (3x50 mL). Organic extracts were washed with 2x50 mL $NaHCO_3$ saturated solution, 3x50 mL water, dried and concentrated, resulting 2.72g methyl oleate as colorless liquid (yield 92%), ^{1}H-NMR ($CDCl_3$, δ ppm, *J* Hz): 5.36-5.33 (m, 2H, H-9, H-10); 3.67 (s, 3H, CH_3O); 2.30 (t, 2H, H-2, 7.4); 2.04-1.96 (m, 3H); 1.66-1.57 (m, 3H); 1.36-1,23 (m, 20H); 0.88 (t, 3H, H-18), ^{13}C-NMR ($CDCl_3$, δ ppm); 174.47 (C-1); 130.14, 129.89 (C-9, C-10), 51.64 (CH_3O); 34.25 (C-2), 32.05 (CH_2); 29.91, 29.82, 29.67, 29.46, 29.29 ($9CH_2$), 27.36 (CH_2), 25.09 (CH_2), 22.82 (C-17); 14.24 (C-18); the compound has the same characteristics with those mentioned in the literature [6].

b. Synthesis of N-[2-(4-methoxyphenyl)ethyl]oleamide

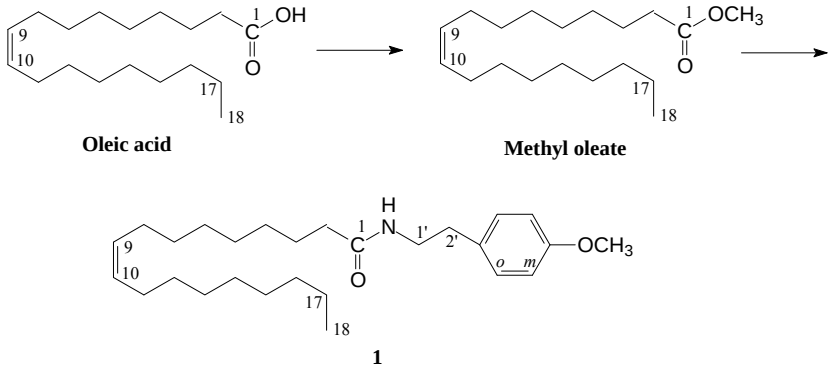
5.6g (5.4mL, 0.037 moles) of 2-(4-methoxyphenyl)ethylamine, 10g (0.033moles) methyl oleate, 100 mL anhidrous methanol and 10 mL 29% (w/w) sodium metoxide in methanol were introduced in a 250 mL round bottom flask, and refluxed under stirring for 8 hours monitoring the reaction by TLC on Merck silica gel plates, with ethyl acetate-methanol, 90:13, eluent system; $R_{f,\ methyl\ oleate} = 0.80$, $R_{f,\ amine} = 0.07$, $R_{f,\ compound\ \mathbf{1}} = 0.72$. After cooling to the room temperature, the reaction mixture was acidulated with 35 mL 10% HCl solution, then methanol was distilled under reduced pressure. 75 mL water and 200 mL CH_2Cl_2 were added to the concentrate and the phases were separated; the organic phase was washed with 50 mL $NaHCO_3$ saturated solution, dried and concentrated. The

crude product (13g) was purified by pressure chromatography on a silica gel column prepared in extraction gasoline and eluted with extraction gasoline-ethyl acetate (5:1), resulting 10.25 g (yield 68 %) pure compound **1**. **1** was recrystallized from hexane (m.p.= 71.7-73.8°C) as yellow crystals. IR: 3009m ($\nu_{=C-H}$), 2958w, 2919 vs ($\nu CH_{2\ asim}$); 2850s ($\nu CH_{2\ sim}$), 1638 (νC=ONHR sec. amide), 1614w, 1584s (νCONH band II), 1548s (νC=O sec. amide, band II), 1514m, 1246s (ν=C-O-CH_3); 1197w; 1186w; 1031w; 820w, ^{1}H-NMR ($CDCl_3$, δ ppm, *J* Hz), 7.09 (d, 2H, H-*o*, 8.5); 6.84 (d, 2H, H-*m*, 8.5); 5.53 (br.s., 1H, NH); 5.35-5.31 (m, 2H, H-9, H-10); 3.78 (s, 3H, CH_3O); 3.64 (q, 2H, H-1', 6.9); 2.74 (t, 2H, H-2', 6.9); 2.10 (t, 2H, H-2, 7.5); 2.05-1.94 (m, 3H); 1.63-1.52 (m, 3H); 1.56-1.21 (m, 20H); 0.87 (t, 3H, H-18), ^{13}C-NMR ($CDCl_3$, δ ppm): 173.18 (C-1); 158.37 (C_q-OMe); 131.02 (C_q), 130.08, 129.83 (C-9, C-10), 129.77 (2C-*o*), 114.12 (2CH, C-*m*); 55.33 (CH_3O), 40.78 (CH_2, C-1''); 36.92 (CH_2, C-2''); 34.91 (C-2); 31.97 (CH_2); 29.86, 29.82, 29.61, 29.41, 29.36, 29.23, 27.32, 27.27, 25.85 ($9CH_2$), 22.77 (C-17), 14.20 (C-18).

3. Results and Discussion

3.1. Synthesis

Synthesis of amides like anandamide [7], prostamides [8], fatty acids amides [9], macamides, could be realized by several methods: direct esterification of acids with amines [10] in the presence of carbodiimide [11], chlorophormiate [12], or with carbonyldiimidazole [5], acid chloride and amines [13], esters with amines catalyzed by bases like MeONa [14]. We realized the synthesis of oleamide compound **1** according to [14], as seen in Scheme 1:



Scheme 1: Synthesis of compound **1**

Methyl oleate was obtained by esterification according to usual methods. It was then reacted in the presence of sodium methoxide as catalyst with 2-(4-methoxyphenyl)ethylamine in anhydrous methanol at 60°C. After column chromatography purification the pure compound **1** resulted in a 68 % yield. The

recrystalized fraction from hexane was used for physico-chemical and electrochemical characterization of the compound.

3.2. Physico-chemical characterization

3.2.1. IR and NMR

IR spectrum of the compound **1** presents a very strong band at 1638 cm^{-1} and two intense bands at 1584 and 1548 cm^{-1} characteristics for secondary amides, a strong band for phenolic methyl ether at 1246 cm^{-1} and also intense bands for oleyl fragment of the molecule (the entire IR spectrum is given in the experimental part).

In the proton spectrum of compound **1** the signals for the linked amidic fragment at 5.53 (br.s.) are clearly distinguished for all protons: ethylene at δ 3.64 (H-1') and 2.74 (2') ppm, methyl ester at 3.78 ppm and aromatic group at 7.09 (H-*o*) and 6.84 (H-*m*) ppm. The same remark available for carbon atoms, both spectra confirming the structure of the molecule.

3.2.2. Mass spectra

For the identification of p-methoxy-phenetyl-oleylamide, MS obtained at high resolution was compared with the simulated one for a compound with the formula $C_{27}H_{46}NO_2$, in its protonated form $[M+H]^+$. This compound revealed a spectrum with peaks at m/z 416.35, m/z 417.35 and m/z 418.35, corresponding to isotope peaks at $[M+H]^+$, $[M+1+H]^+$ and $[M+2+H]^+$.

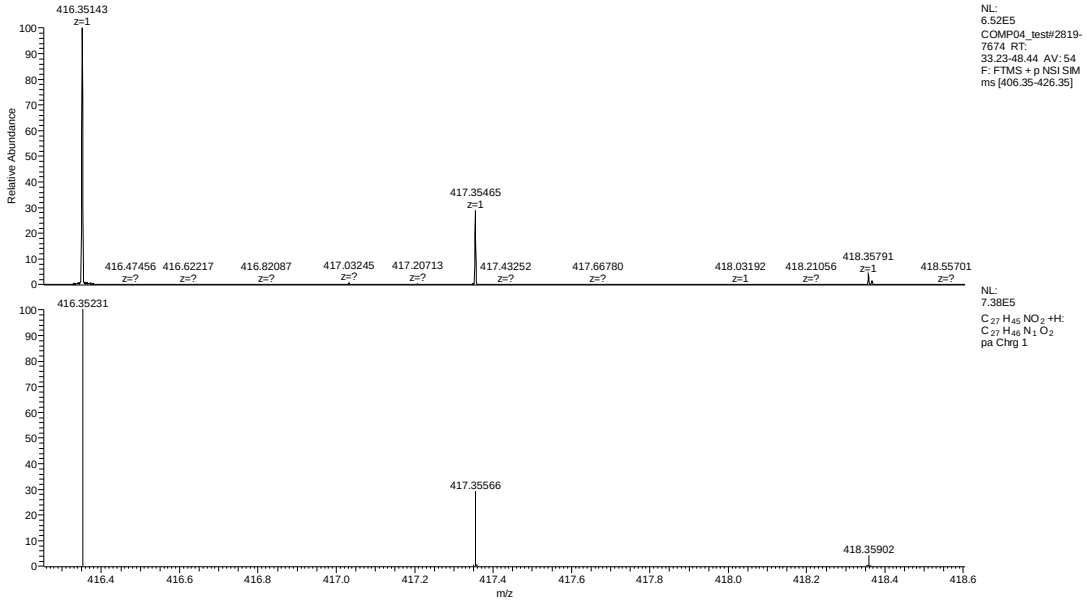


Fig.2. Experimental MS for N-[2-(4-methoxyphenyl)ethyl]oleamide (up) and simulated isotopic pattern of $[M+H]^+$ for a compound with the molecular formula $C_{27}H_{46}NO_2^+$ (down)

Fig. 2 reveals in the upper part the MS experimentally obtained at high resolution and in the lower part the simulated isotopic pattern for the compound **1**. The pattern is similar regarding m/z of the main peaks observed and the relative abundance of the $[M+1+H]^+$ peak (~ 29 %).

To further validate our identification we fragmented the precursor by using collision induced dissociation (CID). The fragmentation spectrum of m/z 416.35 reveals the presence of ions with m/z 152 and m/z 265 corresponding to fragmentation of the amide group. Also, the peaks with m/z 135 and m/z 282 corresponding to fragmentation of alkyl amide group were detected. Minor fragments of consecutive olefinic backbone fragmentation are also observed, the corresponding ions being separated by 14 Da (CH_2) (Fig. 3).

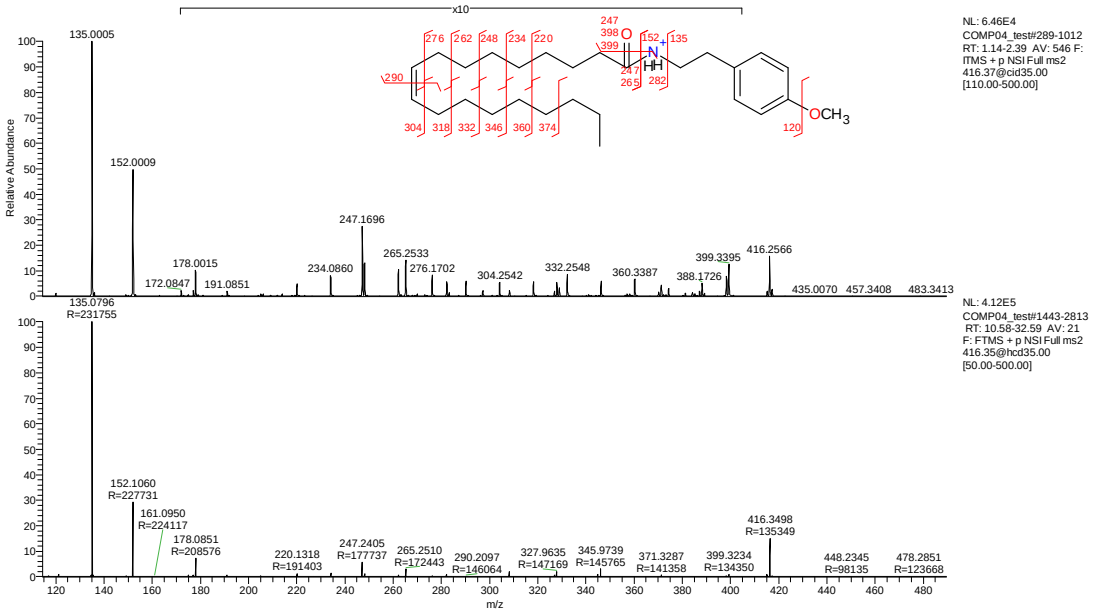


Fig. 3. MS/MS CID fragmentation (up) and HCD fragmentation (down) spectra of ion m/z 416.35

The isotopic pattern obtained for compound **1** corresponds to the empirical formula ($C_{27}H_{46}NO_2$). Fragmentation of this precursor yields a series of cluster ions (spaced 14 Da apart) specific for the fragmentation at the oleyl chain. Major peaks are found in the MS/MS fragmentation, corresponding to the cleaveage at the amide group.

3.3. Electrochemical characterization

The electrochemical behavior of **1** was studied on a stationary electrode in acetonitrile (CH_3CN) containing tetrabutylammonium perchlorate (TBAP) as supporting electrolyte, using a glassy carbon electrode.

The electrochemical experiments were carried out by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). Anodic and cathodic curves were recorded individually, starting from the stationary potential. CV and DPV curves were recorded for various concentrations (0-3mM) of the studied compound in 0.1M TBAP in CH_3CN. The DPV curves obtained for different concentrations of compound **1** are presented in figure 4. Three anodic (1a-3a) processes are observed, denoted in the order in which they appear in the voltammograms. No cathodic process can be evidenced. The CV curves for increasing concentrations of compound **1** are shown in Fig. 4.

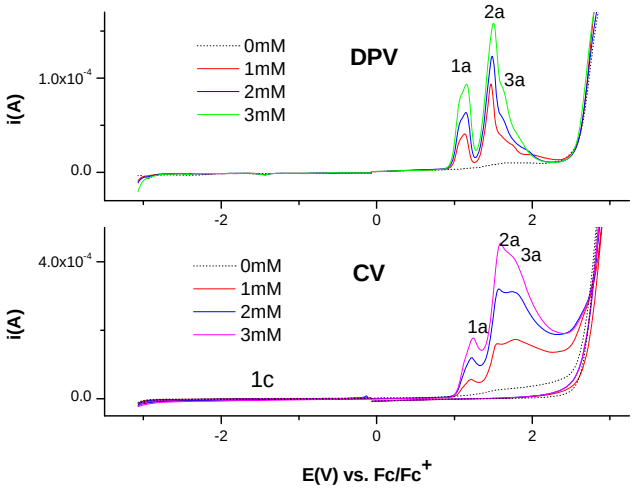


Fig. 4. DPV and CV (0.1 V/s) curves for different concentrations of **1** in 0.1 M TBAP, CH_3CN on glassy carbon electrode (3 mm diameter)

Both DPV and CV curves show peak currents linearly increasing with the concentration (plots up and down on the left of Fig. 5). The peak potentials linearly increase with the logarithm of concentration (plots up and down on the right of Fig. 5).

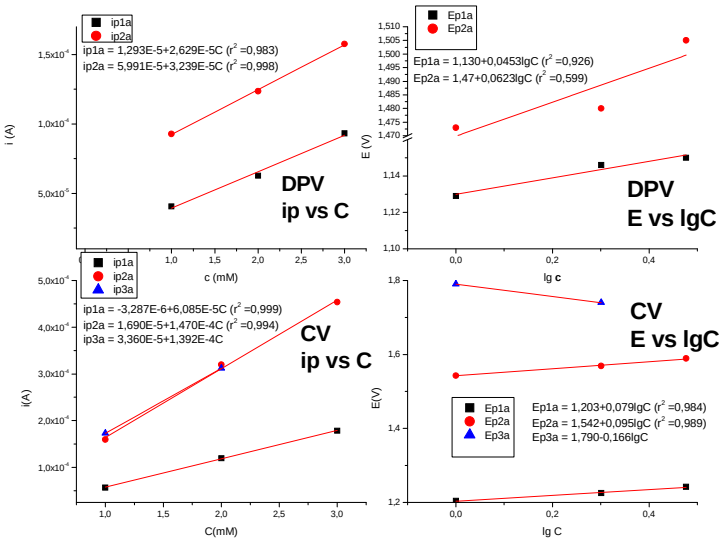


Fig. 5. Dependences on concentration of the currents (up and down on the left) and potentials (up and down on the right) of the peak shown in Fig.4

The influences of the scan rate and scan domain on the CV curves are presented in Fig. 6 (A and B, respectively). All processes are irreversible. The first peak current linearly increases with the square root of the scan rate showing a diffusion controlled process. The diffusion coefficient can be estimated.

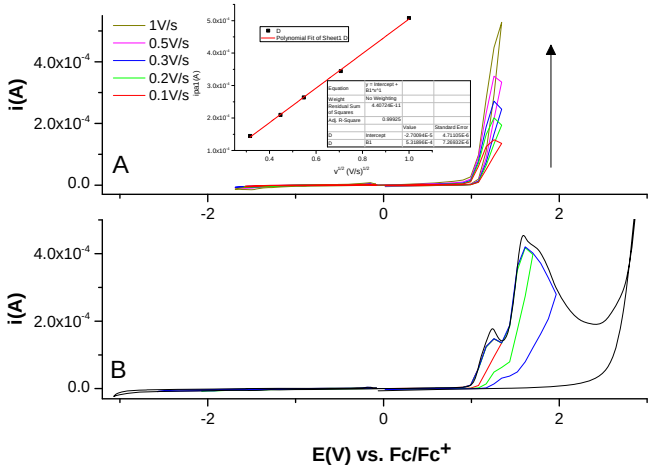


Fig. 6. CV curves at different scan rates (A): 0.1; 0.2; 0.3; 0.5; 1V/s in the domain of the peak 1a, and for various scan domains (B) in the domains of the peaks 1c and 1a at 0.1V/s obtained on glassy carbon (3 mm diameter) in (3 mM) solution of **1** in 0.1 M TBAP, CH_3CN

By cycling the potential in the range of the first anodic peak a decrease of the current can be seen (Fig.7), showing the formation of a film or accumulation of insoluble products on the electrode surface. The cycling led to a modified electrode.

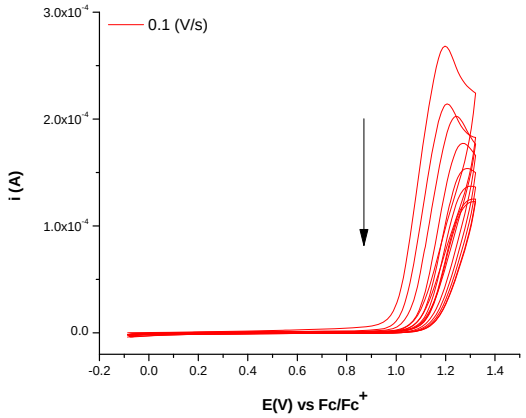


Fig. 7. Successive scanning (20 cycles) with 0.1 V/s

The transfer of this modified electrode in pure electrolyte shows altered CV curves for a redox probe (ferrocene). The anodic peak current for Fc/Fc^+ couple is less influenced in comparison with the cathodic peak current which is smaller than that for the bare electrode (Fig. 8). The difference between the peak potentials of ferrocene is bigger (0.144V) for the modified electrode than for the bare electrode (0.083V), confirming the electrode covering with a insulating layer (film or insoluble products).

The electrode modification can be performed also by controlled potential electrolysis.

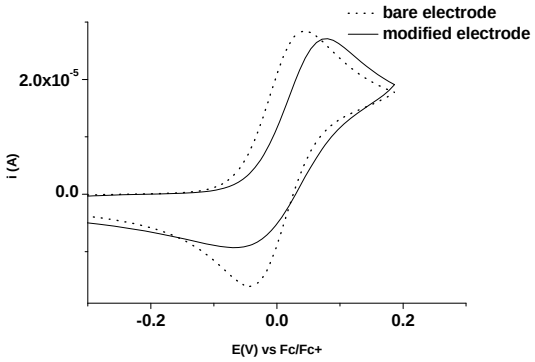


Fig. 8. CV curves in 1mM ferrocene solution in **1** (3mM) in 0.1M TBAP, CH_3CN for the modified electrode prepared by scanning (20 cycles) as shown in Fig. 7

4. Conclusions

The new oleylamide compound **1** was synthesized from oleic acid in a sequence of two reactions. The compound was purified by pressure chromatography and recrystallized from hexane. The physico-chemical characterization by IR, MS and ^{1}H- and ^{13}C-NMR spectra confirmed the structure of the compound. Three anodic irreversible processes were identified by cyclic and differential pulse voltammetry. Scanning the potential in the range of the first anodic peak led to the coverage of the electrode with an insulating layer.

Acknowledgements

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