Electrochemical Chlorination of Physcion – An Approach to Naturally Occurring Chlorinated Secondary Metabolites of Lichens

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The electrochemical chlorination of physcion (=1,8-dihydroxy-3-methoxy-6-methylanthracene-9,10-dione; **1**) in AcOH and CH_2Cl_2 was investigated by cyclic voltammetry and prep.-scale electrolysis. This approach provided access to a number of diverse biologically and pharmacologically interesting chlorinated secondary metabolites of lichen. Unlike the only previous literature report on the 'classical' chlorination of physcion (**1**), which allowed the preparation of 4-chlorophyscion (**2b**), 4,5-dichlorophyscion (**3b**), and 2,4,5-trichlorophyscion (**4**), the present procedure also gave fragilin (=2-chlorophyscion; **2a**) and 2,4-dichlorophyscion (**3a**), alongside the previously obtained **2b**, **3b**, and **4**. All of these compounds, except for **2a**, were isolated by column chromatography and medium-pressure liquid chromatography (MPLC) and characterized by spectral data. The preparative electrolysis with a 2 $F \cdot mol^{-1}$ charge consumption in AcOH and 10 $F \cdot mol^{-1}$ in CH_2Cl_2 may have a practical synthetic utility, since the thus obtained product mixtures can be readily fractioned by column chromatography to afford pure **2b** and **4**, respectively. The regioselectivity of the reaction is explained by the resonance stabilization of the corresponding arenium cations - potential products of an electrophilic attack of a 'positive' Cl species on the physcion molecule.

Introduction. – The scientific interest in secondary metabolites produced by plants has, nowadays, increased due to an intense search for new drugs of plant origin. The extensive research performed in this field made secondary metabolites an important source of bioactive compounds. For example, miscellaneous antraquinone (=anthracene-9,10-dione) derivatives, such as physcion (often called parietin), emodin, fallacinal, teloschistin, chrysophanol, xanthorin, *etc.*, abound in lichens and some plants [1], from which they can be easily isolated. In many cases, isolated single compounds of this kind, as well as the whole plant extract possess bioactivity, so that a plethora of research reports devoted to this problem can be found in the literature [2–9]. Halogenated antraquinones, like fragilin, 2-chloroemodin, *etc.*, also appear among secondary metabolites of some lichens, although as minor components [10][11].

A partial, selective transformation of a naturally occurring compound is very often the best way to synthesize another one with a similar structure, that is, however, present in less quantity or absent from the corresponding natural source. Even more, in some cases, this is the only economically feasible way to synthesize compounds that could be

interesting for certain purposes. Thus, to obtain two of the above mentioned natural chlorinated antraquinones, fragilin (=2-chloro-1,8-dihydroxy-3-methoxy-6-methylanthracene-9,10-dione; **2a**) and 2-chloroemodin (=2-chloro-1,3,8-trihydroxy-6-methylanthracene-9,10-dione), *Sargent* and co-worker [12] chlorinated physcion (=1,8-dihydroxy-3-methoxy-6-methylanthracene-9,10-dione; **1**) isolated from *Xanthoria parietina* (L.) BELTRAM, and then selectively dechlorinated the obtained trichloro derivative. In continuation of our permanent interest in the electrochemical halogenation of natural products or their derivatives [13–16], we decided to examine the possibility of an electrochemical chlorination of physcion (**1**), the most widespread anthraquinone derivative in lichens [4]. At least, two advantages of an electrochemical approach compared with the classic one exist: *i*) it is much more appropriate to handle a chloride (from which free Cl_2 will be liberated through this process) than harmful gaseous Cl_2 stored in bottles under pressure and *ii*) the electrochemical method can provide a much more precise Cl_2-dosage control. The latter problem is not an insignificant one, particularly when working with small amounts of (expensive) substrates, and this is very often the case when studying certain natural products (including those described in the present work). The preparation of a solution of Cl_2 in the corresponding solvent and determination of the exact concentration of 'active' Cl_2 by classical analytical methods is the usual way to overcome this problem. An electrochemical method, however, allows for the addition (liberation) of free Cl_2 into the reaction mixture practically molecule by molecule. We now wish to report on our first results obtained through both the cyclovoltametric measurements and the preparative scale electrolysis of physcion (**1**) isolated from a lichen species of the genus *Xanthoria* by a known method [12].

Results and Discussion. – The electrochemical chlorination of physcion (**1**) was conceptualized as the electrolysis of a chloride in the presence of substrate **1** and the appropriate system of solvent and electrolyte. Since the classical chlorination of **1** described in [12] has been performed in AcOH and $CHCl_3$ as the solvents, we chose Et_4NCl in AcOH and CH_2Cl_2 as the electrolysis media (the use of $CHCl_3$ as the solvent in preparative-scale electrochemical experiments is not suitable because its salt solutions do not provide reasonable electrical conductivity). However, we performed several cyclovoltammetric experiments before the preparative electrolysis to examine the electrochemical behavior of the chloride and physcion in these solvents with $Bu_4N(ClO_4)$ (TBAP) as the electrolyte. The substrate **1** and the chloride ions were first analyzed separately, and then the reactivity of the species generated by the oxidation of the chloride was evaluated by cyclovoltammetry of these ions in the presence of **1**.

On the basis of preliminary measurements, we chose the 0.50–1.65 V potential window for cyclovoltammetry in AcOH. As it can be seen (*Fig. 1, b*), physcion (**1**) is not electroactive in this potential window at a Pt electrode in a 0.1M solution of $Bu_4N(ClO_4)$ in this solvent. The chloride ions of $5 \cdot 10^{-3}$ M Et_4NCl, on the other hand, exhibit one oxidation wave on the forward potential sweep (1.416 V) and one reduction wave (0.893 V) on the back potential sweep under the same conditions (*Fig. 1, c*). This redox couple is due to the oxidation of the Cl^- ion to elemental Cl_2 by the forward potential sweep, and the reduction of Cl_2 to Cl^- at the back potential sweep. The cyclic voltammetry of the chloride Et_4NCl in the presence of **1** ($2.5 \cdot 10^{-3}$ M) results in a

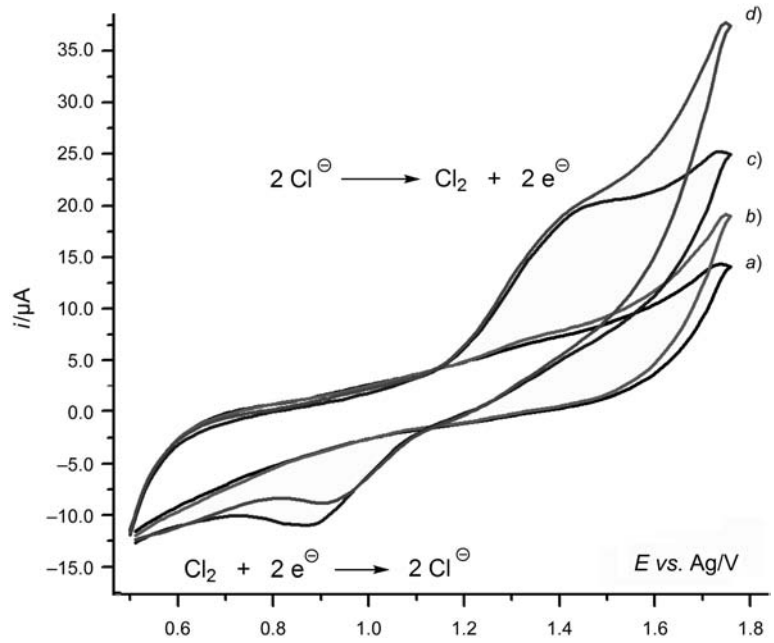


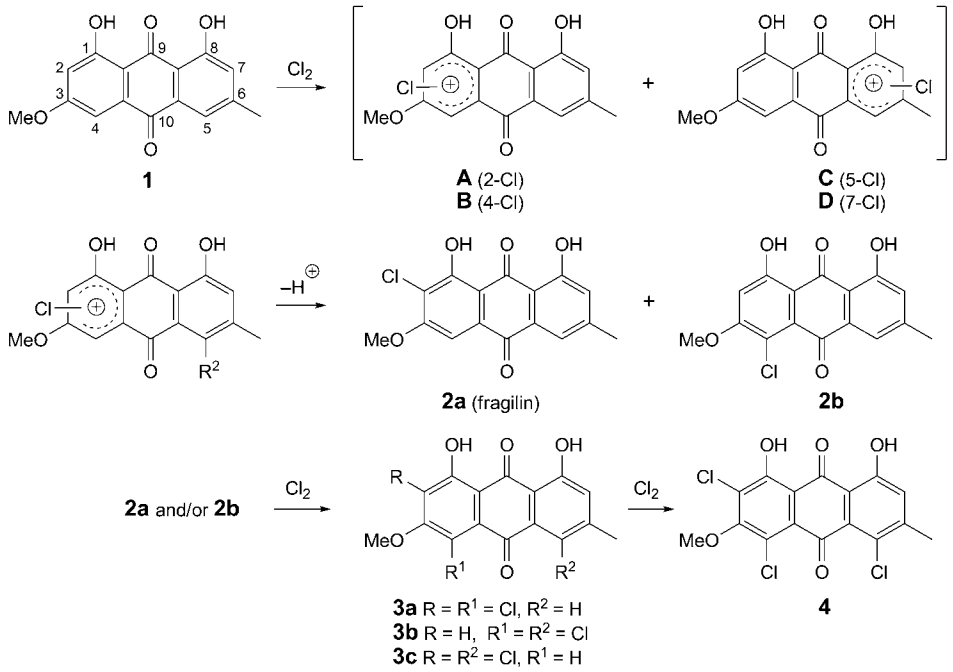
Fig. 1. *Cyclic voltammetry of Et_4NCl and physcion* (**1**) *in 0.1*M *$Bu_4N(ClO_4)$ in AcOH by using a platinum-disc electrode* (2 mm diameter)*, at* v = *0.1 V s^{-1}:* a) *electrolyte $Bu_4N(ClO_4)$,* b) *physcion* (**1**)*,* c) *5 m*M *Et_4NCl,* d) *5 m*M *Et_4NCl/2.5 m*M *physcion* (**1**)

change of the voltammogram shape of these ions, decreasing remarkably the reduction peak current (*Fig. 1,d*). We assume that this decrease is the consequence of the Cl_2-concentration decrease at the electrode surface and its vicinity due to its reaction with **1** (*Scheme*), compared with the experiments without the substrate.

The cyclic voltammetry of the chloride Et_4NCl and **1** in 0.1M $Bu_4N(ClO_4)$ in CH_2Cl_2 was similar to that performed in AcOH, but it was 'cleaner', *i.e.*, the oxidation and reduction peaks were sharper (at 1.200 and 0.780 V, resp., *Fig. 2*). Therefore, exactly the same conclusions as in the previous case can be made by analyzing these results.

Having in mind the above-established facts, we decided to conduct the preparative electrolysis of physcion (**1**) in solutions of Et_4NCl in AcOH and CH_2Cl_2. First we performed a constant-current electrolysis (30 mA) with a 2 $F \cdot mol^{-1}$ charge consumption (the theoretically necessary amount for 1 mol-equiv. of Cl_2, *i.e.*, for the monochlorination) in AcOH, using an undivided electrolytic cell supplied with a cylindrical Pt foil (as the anode) and a spiral Cu wire (as the cathode). Because of the low solubility of **1** in AcOH, this electrolysis was performed at 50°. This resulted in a mixture containing mainly the unconsumed substrate **1**, followed by two products (*Table*, *Entry 1*). The ^{1}H-NMR spectra of this mixture in combination with the GC/MS analysis allowed us to identify these products as fragilin (=2-chlorophyscion; **2a**) [11] and 4-chlorophyscion (**2b**) [12] (*Scheme*). Apparently, a great part of the liberated Cl_2 migrates during the electrolysis to the cathode undergoing the reduction there, causing such a low current efficiency (33%). The mixture containing **1**/**2a**/**2b** was submitted

Scheme. *Electrochemical Chlorination of Physcion* (**1**)



again to electrolysis under the same conditions consuming an additional 4 F mol^{-1} charge, which resulted in a mixture containing the monochlorinated **2a** and **2b** as the main products (*Entry 2*).

Table 1. *Electrochemical Chlorination of Physcion* (**1**)

Entry	Reaction conditions (solvent/cell/F · mol^{-1})	Product distribution [%][a]						
		1	**2a**	**2b**	**3a**	**3b**	**4**	unidentified
1	AcOH/undivided/2	67.0	4.0	29.0	–	–	–	–
2	AcOH/undivided/6	3.7	12.7	77.2	1.5	4.1	–	0.8
3	AcOH/divided/2	4.0	12.5	79.5	–	4.0	–	–
4	AcOH/divided/4	–	3.6	33.5	11.0	19.5	18.7	13.7
5	AcOH/divided/6	–	–	4.3	14.2	39.3	21.4	20.8
6	AcOH/divided/10	–	–	3.7	13.3	21.6	22.8	38.6
7	CH_2Cl_2/undivided/2	96	–	4.0	–	–	–	–
8	CH_2Cl_2/undivided/6	–	17.4	59.8	2.0	15.8	–	5.0
9	CH_2Cl_2/divided/2	30.5	9.1	56.4	3.5	< 1	–	< 1
10	CH_2Cl_2/divided/4	–	14.5	39.0	15.4	16.5	7.4	7.2
11	CH_2Cl_2/divided/6	–	5.9	9.4	10.8	15.5	23.3	35.1
12	CH_2Cl_2/divided/10	–	–	–	–	–	63.5	15.8

[a]) On the basis of ^{1}H-NMR spectra.

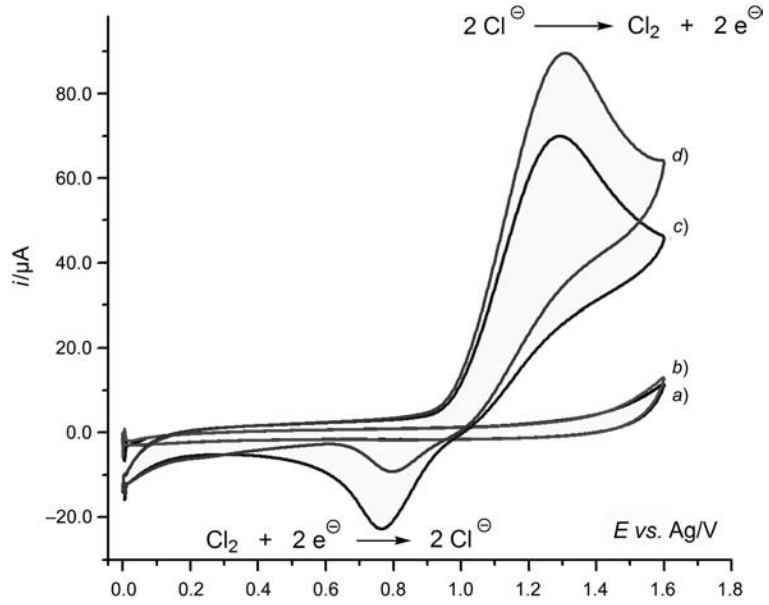


Fig. 2. *Cyclic voltammetry of Et_4NCl and physcion* (**1**) *in 0.1*M *$Bu_4N(ClO_4)$ in CH_2Cl_2 by using a platinum-disc electrode* (2 mm diameter), *at* v = *0.2 V s^{-1}:* a) *electrolyte $Bu_4N(ClO_4)$,* b) *physcion* (**1**), c) *5 m*M *Et_4NCl,* d) *5 m*M *Et_4NCl/2.5 m*M *physcion* (**1**)

However, the starting substrate **1** was not consumed completely although two dichloro derivatives, 2,4- and 4,5-dichlorophyscion (**3a** and **3b**, resp.), appeared as additional reaction products (*Entry 2*). This prompted us to perform a constant-current electrolysis of **1** in the same solvent/electrolyte system but in a divided electrolytic cell (by a ceramic membrane; 20 mA). Thus, the reaction performed at 50°, passing 2 F · mol^{-1} of charge, gave the monochlorinated derivatives **2a**/**2b** in the ratio 13.6 : 86.4, followed by a much smaller amount of unchanged **1** and 4,5-dichlorophyscion (**3b**) (*Entry 3*); therefore, the current efficiency of this reaction is 100%, regardless of the fact that **1** was not completely consumed. We also found that, despite the low solubility of **1** in AcOH, this reaction could be run at room temperature. Indeed, as the electrolysis progressed, the starting suspension of **1** in AcOH became a clear solution, apparently due to a higher solubility of the products (chlorinated physcions) in this solvent than that of **1**.

Electrolysis with a 4 F · mol^{-1} charge consumption in a divided electrolytic cell was also conducted to examine the possibility of a direct dichlorination of physcion (**1**). However, although the two dichlorophyscions **3a** and **3b**, and even 2,4,5-trichlorophyscion (**4**) appeared as the products of this reaction, **2b** was still the most abundant component of the resulting mixture (*Entry 4*). A prolonged time of the electrolysis caused a decrease in the **2b** abundance and an increase of the relative amounts of the dichloro and trichloro derivatives **3a**, **3b**, and **4**. For example, the electrolysis accompanied by a passage of 6 F · mol^{-1} charge through the solution gave a mixture containing traces (less than 0.05%) of **2a**, 4.3% of **2b**, 14.2% of **3a**, 39.3% of **3b**, 21.4%

of **4**, and some unidentified products (*Entry 5*). However, even electrolysis with a 10 $F \cdot mol^{-1}$ charge consumption did not give the pure trichloro derivative **4** (*Entry 6*). The reaction mixtures always contained considerable amounts of the dichloro derivatives **3a** and **3b**. We were able to isolate four of the mentioned products as pure compounds, *i.e.*, **2b**, **3a**, **3b**, and **4**, by combining gravity column chromatography and prep. MPLC. This and the fact that the ^{1}H-NMR spectra of **2a**, **2b**, **3b**, and **4** are known [11][12] enabled us to perform more detailed analyses of the reaction mixtures.

Surprisingly, the electrochemical monochlorination in CH_2Cl_2 as the solvent, in which physcion (**1**) is much more soluble than in AcOH, proceeded with a slightly lower current efficiency and selectivity. Thus, the constant-current electrolysis of **1** in CH_2Cl_2 in an undivided cell (r.t.; 2 $F \cdot mol^{-1}$ charge consumption) resulted in a mixture containing *ca.* 96% of the unchanged **1** and only *ca.* 4% of 4-chlorophyscion (**2b**) (*Entry 7*). On the other hand, the electrolysis in a divided cell under the conditions for monochlorination (2 $F \cdot mol^{-1}$ charge consumption) gave a mixture containing up to 30.5% of unchanged **1** (*Entry 9*). Similarly to the reaction conducted in AcOH, a prolonged electrolysis led to mixtures containing several products. In addition, some new products appeared, but neither the isolation of the pure compounds nor the complete identification through a careful analysis of the ^{1}H-NMR spectra was possible. The electrolysis with a 10 $F \cdot mol^{-1}$ charge consumption, however, resulted in a mixture containing 63.5% of the trichloro derivative **4**, which was isolated by column chromatography for the measurement of its spectral data. This is an additional noteworthy difference between the chlorination in CH_2Cl_2 and AcOH.

Our results considerably differ from those of the only previous report on the chlorination of physcion (**1**) [12], which deserves a short discussion. According to [12], the monochlorination of **1** by Cl_2 itself (1:1 molar ratio of the reactants) gave compound **2b** as the sole product. However, despite the fact that the reaction conditions for monochlorination in our experiments differed, one could assume that the regioselectivity of the monochlorination in both cases would be the same. Since **2b** was isolated by recrystallization in [12], the presence of the unchanged substrate **1** and of fragilin (**2a**) might have been missed because these substances should have remained in the mother liquor. On the other hand, under the conditions of monochlorination (*Table*, *Entries 3* and *9*), the other two possible monochloro derivatives, 5- and 7-chlorophyscion, did not form. We made sure that these latter monochloro derivatives were absent by a careful analysis of the monosubstitution pattern in the ^{1}H-NMR spectra. The introduction of a Cl-atom into the physcion molecule or already chlorinated physicon molecules does not change the chemical shifts of the remaining protons to any significant extent but causes only small differences which, however, allow to distinguish different di-, mono-, and nonchlorinated physcion (compare the data of **3a** in the *Exper. Part* and the literature data of **1**, **2a**, **2b**, **3b**, and **4** [4][11][12]). We assume that the observed regioselectivity of the monochlorination of **1** is the consequence of a somewhat different stabilization of the corresponding intermediary ions (by the positive-charge delocalization) obtained after the initial attack of a 'positive chlorine' species on physcion. Indeed, both of the 'outer' benzene rings of **1** are equally activated by the OH groups and deactivated by the C=O groups for an electrophilic substitution, but the MeO-substituted benzene ring is rather more activated than the Me-substituted one (the predomination of **2b** over **2a**, on the other

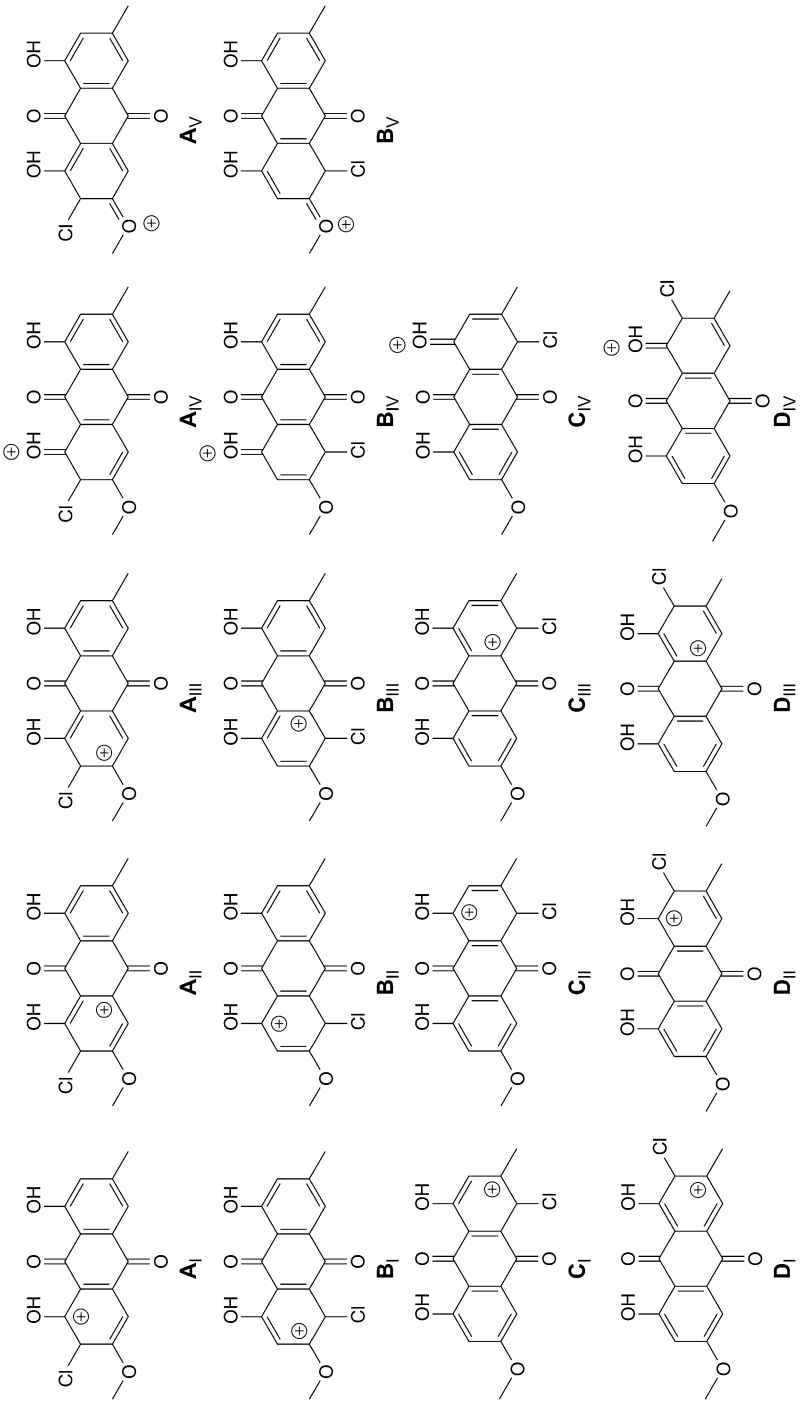


Fig. 3. *Resonance stabilization of possible arenium cations obtained after the electrophilic attack of a 'positive chlorine' species on the molecule of physcion* (**1**)

hand, is steric in nature). As depicted in *Fig. 3*, the corresponding arenium ions obtained on attack of **1** at the positions 2 and 4 (see $\mathbf{A}_{I-V}$ and $\mathbf{B}_{I-V}$) possess one important contributing resonance structure more (see $\mathbf{A}_V$ and $\mathbf{B}_V$) than those obtained by the attack at the positions 5 and 7 (see $\mathbf{C}_{I-IV}$ and $\mathbf{D}_{I-IV}$).

In our experiments the regioselectivity of dichlorination is surely different from that reported for the classical method [12]. Under all conditions permitting dichlorination (electrolysis with 4 or more $F \cdot mol^{-1}$ charge consumption), we found both 2,4- (**3a**) and 4,5-dichlorophyscion (**3b**) in the reaction mixtures. The formation of **3a** rather than **3c** illustrates in the best way how much the MeO-substituted benzene ring is more susceptible to electrophilic attack than the Me-substituted one. Namely, even though the MeO-substituted ring bearing the newly introduced Cl-atom at position 2 (see **2a**) should be deactivated to some extent for further electrophilic substitution on dichlorination by another electrophilic attack, the second Cl-atom is bonded to this ring and not to the unchlorinated one.

Yet, another explanation for the observed regioselectivity of chlorination may be operational. In the case of phenol and 4-chlorophenol in neutral to acidic media, the rate constant for chlorination is up to an order of magnitude higher for the already chlorinated compound [17]. This is explained by the mechanistic consideration that the chlorination of phenol and chlorophenols proceeds in neutral and alkaline media by the reaction of hypochlorous acid with the phenolate or chlorophenolate [17]. Since chlorinated phenols are more acidic than the parent phenols (and this is even more true in the case of the unchlorinated Me-substituted ring of physcion), the concentration of the true nucleophile in this reaction, the phenolate, is greater for the monochlorinated phenols and hence this makes them more reactive in electrophilic aromatic substitutions (a well known example [17] of this is the polyhalogenation of phenol itself with less than 1 equiv. of a halogen available for reaction).

Conclusions. – Electrochemical chlorination of physcion (**1**) in AcOH and CH_2Cl_2 allowed access by avoiding ring synthesis to fragilin (**2a**), 4-chlorophyscion (**2b**), 2,4- and 4,5-dichlorophyscions (**3a** and **3b**, resp.), and trichlorophyscion **4**. The electrolysis with a $2\ F \cdot mol^{-1}$ charge consumption in AcOH and $10\ F \cdot mol^{-1}$ in CH_2Cl_2 may have a practical synthetic utility, since the reaction mixtures obtained under these conditions could readily be separated by column chromatography to afford pure **2b** and **4**, respectively. The pure dichloro derivatives **3a** and **3b** were isolated by prep. MPLC. Electrochemical chlorination of physcion (**1**) presents a good alternative to the classical chlorination of this natural product with free Cl_2 because it avoids the disadvantageous use of gaseous Cl_2 stored in the high-pressure cylinders. To the best of our knowledge, there is no other rich source of chlorinated physcions, thus turning our electrochemical-chlorination procedure into a useful access to these compounds, which will allow to study their properties including their promising biological and pharmacological activities.

This work was supported by the *Ministry of Education and Science of the Republic of Serbia* (grant 172034). We are grateful to *Vida Đuričić* (Technician School 'Nikola Tesla', Leposavić, Serbia) for supplying us with generous amounts of the lichen *Xanthoria elegans.*

Experimental Part

General. The physcion (**1**) used in this study was isolated from a lichen species of the genus *Xanthoria* by a known method [12]. All other chemicals were commercially available and used as received, except for the solvents, which were purified by distillation. $Bu_4N(ClO_4)$ was used as the supporting electrolyte in the cyclic voltammetry experiments. Prep.-scale electrolyses: divided (by a ceramic membrane) or undivided cell in an *Autolab* apparatus; cylindrical Pt foil (2.5 cm diameter) and Pt spiral (1 cm diameter) as the anode and the cathode, resp. Column chromatographic (CC): silica gel *60* (SiO_2; 230–400 mesh ASTM; *Merck*). Prep. medium-pressure liquid chromatography (MPLC): pump module *C-601* and pump controller *C-610*, *Work-21* pump (*Büchi*, Switzerland); pre-packed column cartridges (40 × 75 mm; SiO_2 *60*, particle size distribution 40–63 μm; *Büchi*). TLC: SiO_2 *60* F_{254} (*Merck*) on Al plates, layer thickness 0.2 mm. Cyclic voltammetry: at r.t. under Ar in a three-electrode cell; *Autolab* potentiostat (*PGSTAT 302N*); working electrode Pt disk (2-mm diameter), counter electrode Pt wire, and reference electrode. Ag wire. IR Spectra: *Perkin–Elmer FT-IR 31725-X* spectrophotometer; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Varian-Gemini* (200 MHz) spectrometer; in $CDCl_3$; δ in ppm rel. to Me_4Si as the internal standard, *J* in Hz. EI-MS: mass selective detector *Hewlett–Packard* (*5975B*) part of a GC/MS system (70 eV); in *m/z* (rel. %). GC/MS: *Hewlett-Packard 6890N* gas chromatograph; fused-silica cap. column *DB-5MS* (5% phenylmethylsiloxane, 30 m × 0.25 mm, film thickness 0.25 μm; *Agilent Technologies*, USA) and *5975B* mass selective detector (from the same company); carrier gas He at 1.0 ml min^{-1}.

Prep. Electrolyses. The electrochemical cells (divided and undivided) were filled with AcOH or CH_2Cl_2 (30 ml) containing Et_4NCl (0.1M). Physcion (**1**; *ca.* 30 mg, *ca.* 0.1056 mmol) was then added (in the anodic compartment if the divided cell was used). Electrolyses were performed at a constant current (30 mA in the undivided cell and 20 mA in the divided one). Reactions were run until a charge of 2, 4, 6, or 10 F (*ca.* 20.4, *ca.* 40.8, *ca.* 61.2, or *ca.* 101.9 C, resp.) was passed through the solns. (see *Table*). The solvent was distilled off under reduced pressure from the crude reaction mixture obtained after the end of the electrolysis, the residue extracted with Et_2O, the Et_2O phase washed with the sat. $NaHCO_3$ soln., dried overnight (Na_2SO_4), and concentrated, and the residue separated by CC and MPLC and analyzed by TLC, GC/MS, and NMR. The identification of the products was performed by combining the ^{1}H-NMR and GC/MS data. Compounds **2a**, **2b**, **3b**, and **4** (*Scheme*) are known, and their ^{1}H-NMR data were identical to those given in [11][12].

2,4-Dichlorophyscion (=*2,4-Dichloro-1,8-dihydroxy-3-methoxy-6-methylanthracene-9,10-dione*; **3a**). IR (KBr): 3414.8, 3138.7, 2953.75, 2924.6, 2853.8, 1731.8, 1681.9, 1635.2, 1602.2, 1542.2, 1485.6, 1456.9, 1392.8, 1369.9, 1348.0, 1305.5, 1266.0, 1244.9, 1212.9, 1144.0, 1115.9, 1034.0, 974.4, 912.6, 864.0, 822.2, 812.5, 792.8, 765.0, 756.4, 707.3, 624.2, 588.36, 560.5, 540.6, 478.5. ^{1}H-NMR (200 MHz, $CDCl_3$): 2.48 (*s*, Me); 4.04 (*s*, MeO); 7.11 (*s*, H–C(7)); 7.63 (*s*, H–C(5)); 11.68 (*s*, OH); 13.53 (*s*, OH). EI-MS: 354 (63.5), 352 (100.0, M^+), 335 (2.6, $[M-OH]^+$), 317 (12.5, $[M-Cl]^+$), 245 (2.9), 162 (9.5).

REFERENCES

[1] S. Huneck, I. Yoshimura, 'Identification of Lichen Substances,' Springer-Verlag, Berlin, Heidelberg, 1996.
[2] J. C. Cyong, T. Matsumoto, K. Arakawa, H. Kiyohara, H. Yamada, Y. Otsuka, *J. Ethnopharmacol.* **1987**, *19*, 279.
[3] T. Hatano, H. Uebayashi, H. Ito, S. Shiota, T. Tsuchiya, T. Yoshida, *Chem. Pharm. Bull.* **1999**, *47*, 1121.
[4] N. T. Manojlović, S. Solujić, S. Sukdolak, L. Krsić, *J. Serb. Chem. Soc.* **2000**, *65*, 555.
[5] T. Coenye, K. Honraet, P. Rigole, P. N. Jimenez, H. J. Nelis, *Antimicrob. Agents Chemother.* **2007**, *51*, 1541.
[6] S. K. Agarwal, S. S. Singh, S. Verma, S. Kumar, *J. Ethnopharmacol.* **2000**, *72*, 43.
[7] G.-C. Yen, P.-D. Duh, D.-Y. Chuang, *Food Chem.* **2000**, *70*, 437.
[8] V. Kuete, J. R. Nguemeving, V. P. Beng, A. G. B. Azebaze, F.-X. Etoa, M. Meyer, B. Bodo, A. E. Nkengfack, *J. Ethnopharmacol.* **2007**, *109*, 372.

[9] J. D. D. Tamokou, M. F. Tala, H. K.Wabo, J. R. Kuiate, P.Tane, *J. Ethnopharmacol.* **2009**, *124*, 571.
[10] G. Bendz, G. Bohman, J. Santesson, *Acta Chem. Scand.* **1967**, *21*, 2889.
[11] T. Bruun, D. P. Hollis, R. Ryhage, *Acta Chem. Scand.* **1965**, *19*, 839.
[12] M. V. Sargent, D. Smith, J. A. Elix, *J. Chem. Soc. C* **1970**, 307.
[13] S. Milisavljević, R. D. Vukićević, *J. Serb. Chem. Soc.* **2004**, *69*, 941.
[14] S. S. Milisavljević, K. Wurst, G. Laus, M. D. Vukićević, R. D. Vukićević, *Steroids* **2005**, *70*, 867.
[15] I. Damljanović, M. Vukićević, R. D. Vukićević, *Bull. Chem. Soc. Jpn.* **2007**, *80*, 407.
[16] I. Damljanović, M. Vukićević, D. Manojlović, N. Sojic, O. Buriez, R. D. Vukićević, *Electrochim. Acta* **2010**, *55*, 965.
[17] G. F. Lee, J. C. Morris, *Int. J. Air Water Pollut.* **1962**, *6*, 419.

Received November 17, 2010