THE FORMATION OF FERULIC ACID FROM GAMMA-ORYZANOL HYDROLYSIS UNDER ALKALINE CONDITION USING ULTRASONIC IRRADIATION

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Hoa Thi Truong, Manh Van Do, Long Duc Huynh, Linh Thi Nguyen
Danang Environmental Technology Center, Institute of Environmental Technology,
Vietnam Academy of Science and Technology
Kiyoshi Imamura, Yasuaki Maeda
Research Organization for University
Community Collaborations, Osaka Prefecture University
Ngo Dinh Vu
Viet Tri University of Industry

TÓM TẮT

NGHIÊN CỨU ĐIỀU CHẾ AXIT FERULIC TỪ QUÁ TRÌNH THỦY PHÂN GAMMA ORYZANOL TRONG MÔI TRƯỜNG KIỀM VỚI SỰ HỖ TRỢ CỦA SONG SIÊU ÂM

In this study, we investigated the formation of ferulic acid, one of potential antioxidants, medicines, and cosmetics, by the hydrolysis of γ-oryzanol contained in by-products of rice bran oil processing. Using a base-catalysed reaction, the hydrolysis of γ-oryzanol was examined, and the experiments were conducted with various conditions of catalyst amounts, temperature dependency, and ultrasonic frequencies. After 3 hours of conventional heating, 45% and 73% yield of ferulic acid was obtained using a 10:1 (wt/wt) ratio of KOH/ γ-oryzanol at the temperature of 60, and 75 ℃, respectively. The different frequencies (26, 78 and 130 kHz) were investigated to examine effect of ultrasound on yield of hydrolysis. Among them, the uses of 78 and 130 kHz irradiation accelerated the formation of ferulic acid up to 1.6 - fold times greater than that by the conventional heating at 60 ℃, and that yield was increased only 1.2 times when using lower frequency (26 kHz) at same temperature. Utilizing ultrasonic irradiation (78 and 130 kHz) at 75 ℃, the hydrolysis of γ-oryzanol proceeded quantitatively, and the yield of ferulic acid was achieved more than 90%.

Keywords: *ferulic acid, γ-oryzanol, ultrasound, base-catalysed reaction, hydrolysis*

1. INTRODUCTION

Ferulic acid (FA) is a kind of antioxidants widely used in cosmetic industry in term of UV-protection, in food industry as additives, and deeply applied in medical field. As we know, nitrites, normally sodium nitrite, are used in food industry as a kind of preservatives to maintain color and prevent pathogens' growth. In the acidic stomach, nitrites can react with many others compounds to produce nitrosamines, a known carcinogen. Ferulic acid has been investigated to block the formation of nitrosamine from nitrites [1]. Besides, FA also helps to prevent cardiovascular disease, diabetes, Alzheimer, colon cancer disease and reduce blood cholesterol, etc [2-9]. It also reduces the cholesterol levels in serum and liver, protects against coronary disease, various inflammatory diseases [10], act as antimicrobial and anti-inflammatory agents [8].

FA was firstly chemically synthesized in 1925; however its biological effects started to be noticed in 1970s when Japanese researchers discovered the antioxidant properties of FA steryl esters extracted from rice bran oil. The ability as antioxidants is explained by its phenolic nucleus and conjugated double which easily generates the stabilized phenoxy radical.

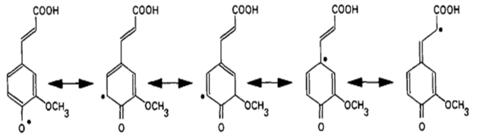

Figure 1. The stable radical of ferulic acid

When any reactive radical attacks ferulic acid, a hydrogen atom will be abstracted to form a phenoxy radical. As shown in Fig. 1, this radical is highly resonance stabilized since the unpaired electron may be present not only on the oxygen but it can be delocalized across the entire molecule. Additional stabilization of the phenoxy radical is provided by the extended conjugation in the unsaturated side chain. This resonance stabilization accounts for the ease of the formation of the phenoxy radical and its consequent lack of reactivity; therefore, this phenoxy radical is unable to initiate or propagate a radical chain reaction [10], therefore FA is an excellent antioxidant. Benzophenones and salicylic acid derivatives are commonly used for UV absorbents; however, some of these derivatives are toxic substances for human beings and the environment. Therefore, nowadays, the friendly environmental UV absorbents compounds are preferred to use such as ferulic acid.

It is well known that ferulic acid can be prepared by the condensation reaction of vanillin with malonic acid in the 40s decade of 21st century. This produces ferulic acid at a high yield, but takes as long as 3 weeks. In addition, the product is a mixture of trans- and cis-isomers, while only trans-ferulic acid, which can be crystallized, has been commercialized. Therefore, the natural sources of ferulic acid are now concerned.

FA can be found in plant tissues such as citrus fruits, banana, eggplant, cabbage, beetroot, broccoli, coffee [10,11], corn bran, rice bran, flax shives, wheat [12], paddy straw [13], *Radix Angelicae sinensi* [14], *Ligusticum chuanxiong* [3], etc. There are many different methods applied to extract FA by alkaline hydrolysis with the support of ultrasound or microwave irradiation [2,3,6,12,15,16]. In general, most researches of extracting FA have had a common basis of using the alkaline hydrolysis, in which depends on the characteristic of raw materials then different conditions were applied. The purpose of this process is to break the organic matrix contained lipids and free fatty acids in the raw materials (plant tissues, cereals); in other hand exploiting the conjugated forms and the hydrolysis of ferulates, esters of ferulic acid to effectively obtain FA. Even though, there are many researches on FA extracting from different raw materials, the basis of hydrolysis process of ferulates hasn't been investigated in details and the final FA content includes both free and conjugated FA.

In cereal, FA is usually located in the bran. It also exists in ester forms such as steryl ferulat which is firstly recognized in 1954 in rice bran oil (RBO). Because these esters were separated from RBO (Orysae Sativa L.) and have one hydroxyl group, they are normally called oryzanol, and γ-oryzanol is the most popular isomers [10]. The γ-oryzanol is a mixture of ferulic esters, of which the four main ferulates are cycloartenylferulate 24-methylene-cycloartanyl ferulate, β-sitosterylferulate and campesterylferulate. From rice bran oil process, the rich source of many nutraceuticals like γ-oryzanol, vitamin E, ferulic acid, and phytic acid is normally lost in the refining process. Instead of being value component in rice bran oil, 96% γ-oryzanol is transferred to the soapstock as a by-product.

Recently, ultrasound-assisted extraction and reaction are prospective and attracted many scientists all over the world [17-22]. There are many researches about ultrasound application for accelerating the ester reaction rate using acid catalyst [23-25], base catalyst [26], with methanol solvent [27-29]; however most esters investigated were methyl esters and there have been few researches related in γ-oryzanol. γ-oryzanol is a commercial product as an excellent antioxidant and reducing plasma cholesterol [30-33]. Due to this reason, γ-oryzanol hydrolysis process hasn't been paid attention yet. However, the hydrolysis process of γ-oryzanol is also not easy because the cholesterol-like groups in its structure is too big that causes steric effect to prevent the reactants attacks to the ester group, thus there needs to have more researches about this hydrolysis reaction.

The aim of this project is to research on the hydrolysis of γ-oryzanol under alkaline condition and to effectively obtain high yield of ferulic acid. The effect of ultrasonic frequencies was also investigated. This research will provide the fundamental data, technology for manufacturing ferulic acid from the by-products (soapstock) of the rice bran oil plant and further applying its antioxidant and anticancer abilities in medicinal purposes.

2. MATERIALS AND METHODS

2.1. Chemicals

Potassium hydroxide, acetic acid and phosphoric acid were analytical grade. The purities of ferulic acid, γ-oryzanol, and ethyl acetate standards (Sigma Aldrich) were more than 95%. Acetonitrile and water are HPLC grade, and other solvents are analytical grade (from Merck).

2.2. Ultrasound equipments

The Kaijo Corporation Ultrasonic Cleaner with 30110 (QR-001) generator and 20202 VS Transducer (26/78/130 kHz) was used. The power output was set at 50 W for all experiments using ultrasonic irradiation. Temperature was controlled by the SANSYO SDT-04 P and AWA 1505 device.

2.3. Quantitative analysis method

The ferulic acid, γ-oryzanol and ethyl

ferulate were analyzed using a reverse phase HPLC column. The HPLC Model GL-7480 (GL Science Inc., Tokyo, Japan) equipped with PDA (GL-7452, GL Science Inc., Tokyo, Japan), and Auto Sampler (GL-7420, GL Science Inc., Tokyo, Japan) was used. The column was a reverse phase of Inertsil ODS-3 C_{18} (3mm x 150 mm, film thickness 3 µm) (GL Science, Tokyo, Japan). The equipment was operated in gradient mode. The mobile phase was a mixture of solvent A (2 mL concentrated H_3PO_4 and 600 mL water) and solvent B (acetonitrile/methanol 1:1). The gradient was conducted as follows: the 30:70 ratio of solvent B to solvent A, gradient to 100:0 for 15 min, hold for 25 min, then gradient back to 30:70 for 5 min, hold for 5 min. The absorbance was monitored at 325 nm for all ferulic acid, ethyl ferulate and γ-oryzanol. Ferulic acid (FA), ethyl ferulate (EF) and γ-oryzanol (Or) contents were calculated based on their own calibration curves with coefficient of determination (R^2) more than 0.999. Yield of ferulic acid was calculated based on following equation:

$$\textbf{yield of FA} = \frac{\textbf{FA}}{\textbf{FA + EF + Or}}$$

2.4. Base-catalysed reaction

The stock solution concentrations of γ-oryzanol in ethyl acetate, aqueous potassium hydroxide were 96 mg/ml and 480 mg/ml, respectively. In order to prepare the reaction, we used ethanol as co-solvent to mix γ-oryzanol/ethyl acetate with aqueous KOH solution as following: 4 ml of γ-oryzanol 96 mg/ml in ethyl acetate + 20 ml of Ethanol + 8 ml of KOH 240 mg/ml. The progress of the reaction was followed at various conditions of different temperature, mass ratio of KOH/ γ-oryzanol and, ultrasonic frequencies. Every 100 µl of sample was taken out at 0 min (right after adding KOH) and after each 30 minutes of all trials. After 3 hours, the reaction was stopped by adding 100 µl of acetic acid and 300 µl of ethanol to 100 µl of sample to form a homogeneous solution. This solution was subject for HPLC analysis.

3. RESULTS AND DISCUSSION

3.1. HPLC separation and the formation of ethyl ferulate

Ferulic acid, ethyl ferulate and γ-oryzanol can be detected by PDA detector at 325 nm, in which ferulic acid peak appears at 1.9 min, ethyl ferulate appears at 9 min and γ-oryzanol is a group of peaks appears from 27.5 min to 33 min, as shown in Fig.2A.

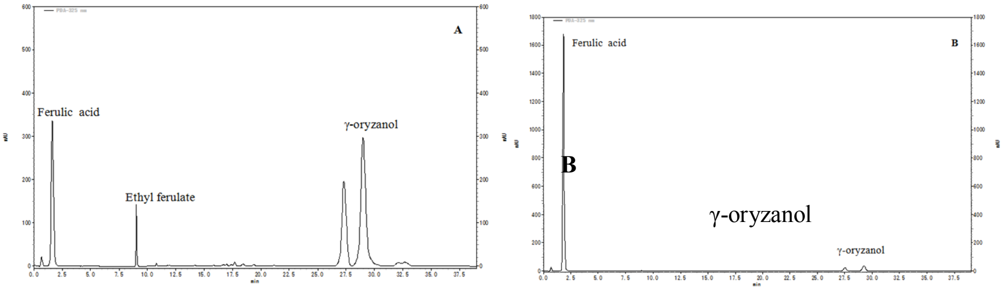

Figure 2. HPLC chromatography separation after A) 30 min; B) 180 min

It was known that there were two competitive reactions including hydrolysis and transesterification process. The less ethyl ferulate was found, the more ferulic acid was formed. In our experiments, the four reactions (1), (2), (3), (4) will be carried out as following:

RCOOR' (Oryzanol) + KOH $\Leftrightarrow$
RCOOK (Ferulate) + R'OH (1)
RCOOR' + $C_2H_5OH \Leftrightarrow RCOOC_2H_5$
(Ethyl ferulate) + R'OH (2)
$RCOOC_2H_5$ + KOH $\Leftrightarrow$ RCOOH
(Ferulic acid) + C_2H_5OH (3)
RCOOK + $CH_3COOH \Rightarrow CH_3COOK$ +
RCOOH (Ferulic acid) (4)

The ethyl acetate content was gradually decreased after 30 min of reaction and not detected as shown in Fig.2B after 180 min of reaction. It means that the transesterification process do not affect to the form of FA after 3 hours despite its competition with hydrolysis reaction from the beginning of reaction.

3.2. Effect of temperature

The base-catalysed reaction was examined at different temperatures ranging of 40, 50, 60, and 75 °C, and yields of ferulic acid were 16, 29, 45 and 73% as shown in Fig.3, respectively, after 3 hours of conventional heating. The achieved results indicated that the higher temperature we used, the higher yield of ferulic acid can be obtained. However, based on the boiling points of solvents used in reaction, the highest temperature used in this experiment was 75 °C.

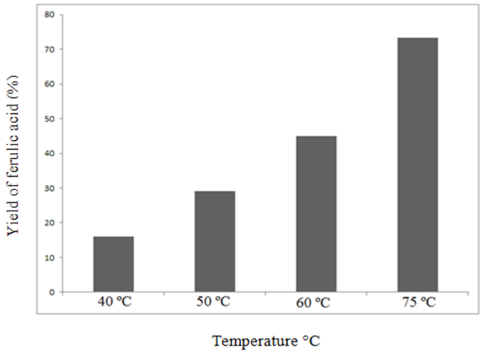

Figure 3. Effect of temperature on ferulic acid yield

3.3. Effect of ratio of KOH/γ-oryzanol

The ratio of KOH/γ-oryzanol was investigated with three values of 10:1, 8:1 and 5:1 (wt/wt). According to Fig.4, the result revealed that the higher ratio of KOH/γ-oryzanol, the higher ferulic acid contents can be obtained. Despite the hydrolysis reaction is followed the stoichiometric ratio of 1:1 (Fig.5), the KOH amount used in our experiments was much higher. There are many researches about ester hydrolysis in base or acid catalyst with ultrasonic assistance but researches about gamma oryzanol hydrolysis is few. The cholesteryl group causes steric effect to stop the conjugated system in ferulic structure by breaking the linear conjugated system, resulting difficulties for OH group to attack -C=O of cacboxyl group. It means that gamma oryzanol is not easily hydrolyzed to form ferulic acid following theoretical molar ratio between the base and the one-cacboxyl group ester. Therefore, in further experiments, we chose the ratio of KOH/ γ-oryzanol as 10:1 for initial γ-oryzanol concentration of 12 mg/mL.

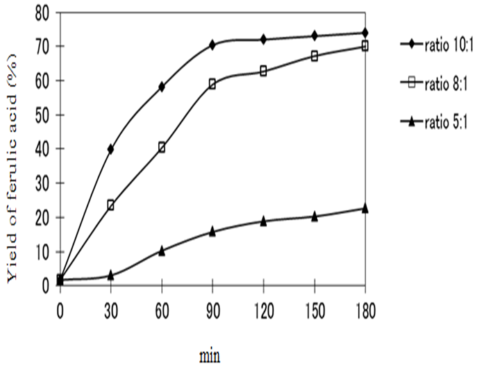

Figure 4. Effect of ratio KOH/oryzanol on ferulic acid yield

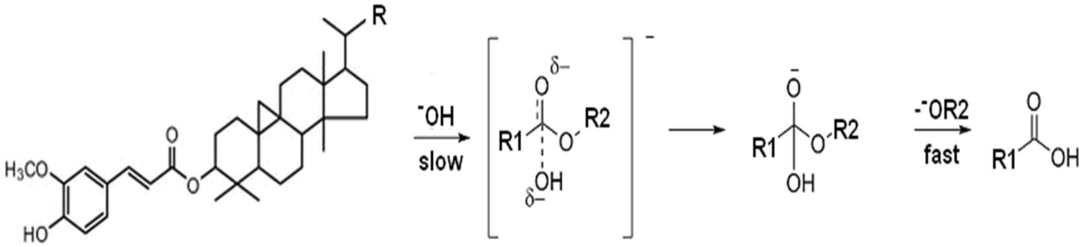

Figure 5. Scheme of hydrolysis reaction of γ-oryzanol

3.4. Effect of ultrasonic frequencies

It is known that ultrasonic irradiation can accelerate the reaction rate of ester hydrolysis. the effect of different ultrasonic frequencies was examined in order to compare with that of conventional heating method at same temperature in this study. The comparison of yield values was implemented between 60 and 75 °C. As shown in Fig 6, the uses of 78 and 130 kHz irradiation accelerated the formation of ferulic acid up to 1.6-fold time greater than that by the conventional heating at 60 ºC, and that yield was increased only 1.2 times when using lower frequency (26 kHz). When temperature was increase up to 75 °C, the hydrolysis of γ-oryzanol proceeded more properly, and the yield of ferulic acid wasreached more than 90% after 3 hrs of reaction. It is concluded that both temperature and ultrasonic frequencies are important factors affected to the yield of hydrolysis reaction of 12 mg/mL γ-oryzanol with KOH/ γ-oryzanol ratio of 10:1 (wt/wt). In comparison to the previous one that reported in [34], from which the hydrolysis process was mentioned that taking place in 8 hrs at 90-100 ºC, 1 atm with the yield of ferulic acid from 70 to 90%, our method is prospective in term of time reduction and temperature.

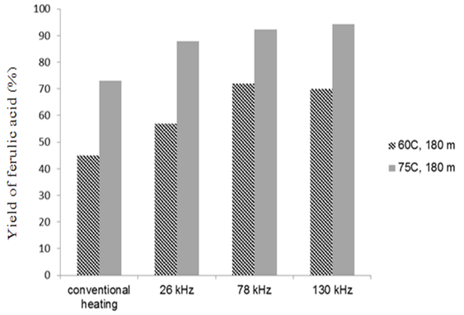

Figure 6. Variation of ultrasonic frequencies in compare with conventional heating method at 60 and 75 °C, 180 min

4. CONCLUSIONS

In this work, the temperature, and the ratio of KOH/γ-oryzanol (wt/wt) are very important factors of the hydrolysis process, which is due to the γ-oryzanol hydrolysis not being followed the normal stoichiometric ratio (1:1) of single ester compound. Ultrasonic irradiation (78 and 130 kHz) accelerated the reaction 1.6 times at 60 °C and the highest yield can be obtained as over 90% by using the assistance of ultrasonic irradiation at 75 °C. This result is a valueable fundamental data for further researches of preparation of ferulic acid from γ-oryzanol containing by-products such as soapstock from rice bran oil processing.

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REFERENCES

1. Itagaki, S., Kurokawa, T., Nakata, C., Saito, Y., Oikawa, S., Kobayashi, M., Hirano, T., and Iseki, K. - In vitro and in vivo antioxidant properties of ferulic acid: A comparative study with other natural oxidation inhibitors, Food Chemistry 114(2009) 466-471.
2. Mussatto, S. I., Dragone, G., and Roberto, I. C. -Ferulic and p-coumaric acids extraction by alkaline hydrolysis of brewer's spent grain, Industrial Crops and Products 25(2007) 231-237.
3. Sun, Y., and Wang, W. -Ultrasonic extraction of ferulic acid from Ligusticum chuanxiong, Journal of the Chinese Institute of Chemical Engineers 39(2008) 653-656.
4. Prasad, N. R., Ramachandran, S., Pugalendi, K. V., and Menon, V. P. -Ferulic acid inhibits UV-B–induced oxidative stress in human lymphocytes, Nutrition Research 27(2007) 559-564.
5. Mancuso, C., and Santangelo, R. -Ferulic acid: Pharmacological and toxicological aspects, Food and Chemical Toxicology 65(2014) 185-195.
6. Yan-Ying, Y., Wei, Z., and Shu-Wen, C. -Extraction of Ferulic Acid and Caffeic Acid with Ionic Liquids, Chinese Journal of Analytical Chemistry 35(2007) 1726-1730.
7. Srinivasan, M., Sudheer, A. R., Pillai, K. R., Kumar, P. R., Sudhakaran, P. R., and Menon, V. P. -Influence of ferulic acid on γ-radiation induced DNA damage, lipid peroxidation and antioxidant status in primary culture of isolated rat hepatocytes, Toxicology 228(2006) 249-258.
8. Saija, A., Tomaino, A., Trombetta, D., De Pasquale, A., Uccella, N., Barbuzzi, T., Paolino, D., and Bonina, F. -In vitro and in vivo evaluation of caffeic and ferulic acids as topical photoprotective agents, International Journal of Pharmaceutics 199(2000) 39-47.
9. Woranuch, S., and Yoksan, R. -Preparation, characterization and antioxidant property of water-soluble ferulic acid grafted chitosan, Carbohydrate Polymers 96(2013) 495-502.
10. Graf, E. -Antioxidant potential of ferulic acid, Free Radical Biology and Medicine 13(1992) 435-448.
11. Zhao, Z., and Moghadasian, M. H. -Chemistry, natural sources, dietary intake and pharmacokinetic properties of ferulic acid: A review, Food Chemistry 109(2008) 691-702.
12. Buranov, A. U., and Mazza, G. -Extraction and purification of ferulic acid from flax shives, wheat and corn bran by alkaline hydrolysis and pressurised solvents, Food Chemistry 115(2009) 1542-1548.
13. Salleh, N. H. M., Daud, M. Z. M., Arbain, D., Ahmad, M. S., and Ismail, K. S. K. -Optimization of alkaline hydrolysis of paddy straw for ferulic acid extraction, Industrial Crops and Products 34(2011) 1635-1640.
14. Liu, Z., Wang, J., Shen, P., Wang, C., and Shen, Y. -Microwave-assisted extraction and high-speed counter-current chromatography

purification of ferulic acid from Radix Angelicae sinensis, Separation and Purification Technology 52(2006) 18-21.

15. Torre, P., Aliakbarian, B., Rivas, B., Domínguez, J. M., and Converti, A. - Release of ferulic acid from corn cobs by alkaline hydrolysis, Biochemical Engineering Journal 40(2008) 500-506.
16. Zhao, S., Yao, S., Ou, S., Lin, J., Wang, Y., Peng, X., Li, A., and Yu, B. - Preparation of ferulic acid from corn bran: Its improved extraction and purification by membrane separation, Food and Bioproducts Processing 92(2014) 309-313.
17. Teh, S.-S., and Birch, E. J. -Effect of ultrasonic treatment on the polyphenol content and antioxidant capacity of extract from defatted hemp, flax and canola seed cakes, Ultrasonics Sonochemistry 21(2014) 346-353.
18. Vilkhu, K., Mawson, R., Simons, L., and Bates, D. -Applications and opportunities for ultrasound assisted extraction in the food industry — A review, Innovative Food Science & Emerging Technologies 9(2008) 161-169.
19. Chemat, F., Zill e, H., and Khan, M. K. -Applications of ultrasound in food technology: Processing, preservation and extraction, Ultrasonics Sonochemistry 18(2011) 813-835.
20. Da Porto, C., Porretto, E., and Decorti, D. -Comparison of ultrasound-assisted extraction with conventional extraction methods of oil and polyphenols from grape (Vitis vinifera L.) seeds, Ultrasonics Sonochemistry 20(2013) 1076-1080.
21. Tao, Y., Wu, D., Zhang, Q.A., and Sun, D.W. - Ultrasound-assisted extraction of phenolics from wine lees: Modeling, optimization and stability of extracts during storage, Ultrasonics Sonochemistry 21(2014) 706-715.
22. Muñiz-Márquez, D. B., Martínez-Ávila, G. C., Wong-Paz, J. E., Belmares-Cerda, R., Rodríguez-Herrera, R., and Aguilar, C. N. - Ultrasound-assisted extraction of phenolic compounds from Laurus nobilis L. and their antioxidant activity, Ultrasonics Sonochemistry 20(2013) 1149-1154.
23. Tuulmets, A., and Salmar, S. -Effect of ultrasound on ester hydrolysis in aqueous ethanol, Ultrasonics Sonochemistry 8(2001) 209-212.
24. Tuulmets, A., and Raik, P. - Ultrasonic acceleration of ester hydrolyses, Ultrasonics Sonochemistry 6(1999) 85-87.
25. Tuulmets, A. -Ultrasound and polar homogeneous reactions, Ultrasonics Sonochemistry 4(1997) 189-193.
26. Nanda, A. K., and Sharma, M. M. - Kinetics of fast alkaline hydrolysis of esters, Chemical Engineering Science 22 (1967) 769-775.
27. M.Khurana, J. -Facial hydrolysis of esters with KOH-Methanol at ambient temperature Monatshefte fur Chemie 135(2004) 83-87.
28. Dayal, B., Salen, G., Toome, B., Tint, G. S., Shefer, S., and Padia, J. - Lithium hydroxide/aqueous methanol: mild reagent for the hydrolysis of bile acid methyl esters, Steroids 55(1990) 233-237.

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