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論　文　名	「Degradation of Phorbol Esters by Plasma, Gamma-ray, Sunlight and UV Irradiations」 （プラズマ、ガンマ線、太陽光および紫外線の照射によるフォルボールエステルの分解）
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論文要旨

　　Bio-diesel, an alternative of petro-diesel, is produced from vegetable oils by a chemical process called transesterification (**Chapter 1**). About 75% of bio-diesel product cost is occupied by the cost of oil feedstock, making bio-diesel still expensive for users. Furthermore, vegetable oil is mostly used as cooking oil, serving vegetable oil as feedstock for bio-diesel production would not be desirable. Recently, the non-edible seed oil from *Jatropha curcas* Linnaeus (JCL) plant has been considered as a promising alternative and the JCL cultivation for biodiesel production purpose has been promoted worldwide. Unfortunately, JCL seed oil is highly toxic due to the presence of biologically active phorbol esters (PEs). These compounds are highly toxic to human through their biologically negative effects such as skin irritancy, inflammation, tumor promotion, etc. There are possibilities of leaching PEs into environment during the process of JCL based biodiesel production. High concentration of PEs (2.08-2.72 mg/g) presenting in wash-water from the pre-treatment steps of JCL oil has been reported and due-care of discarding such PEs-containing water into environment is required. Utilizing PEs as bio-pesticide/insecticide is another concern on the possible ecotoxicology in environment.

Therefore, the assessments of environmental risks of PEs including the study on the degradation of PEs are essential.

The aim of this study is to investigate the degradation of PEs by plasma (electrical discharge), gamma-ray, sunlight and UV light irradiations. In this study, phorbol 12,13-diacetate (PDA), phorbol 12-myristate 13-acetate (PMA) and *Jatropha* phorbol esters (JPEs) were used. Both PDA and PMA are authentic PEs which are commercially available.

The JPEs were obtained by extraction from the kernels of JCL seeds (**Chapter 2**). JCL kernel, brought from T-rang Bang City (the southern area of Vietnam), was used as a JPE source. By pre-established HPLC method, it was found that the JPE content in the JCL seed kernel was about 1.55 mg/g as PMA equivalent. This indicates that the JCL species cultivated in that area is the toxic genotype. JPEs could be successively extracted from JCL kernel by using methanol, followed by hexane-wash, water-wash, and column chromatography to obtain purified JPEs. 12-deoxy-16-hydroxyphorbol esters could be classified as a major group of JPEs in which more than 6 derivatives are present. These compounds possess the same molecular mass of 710, according to mass spectrometric data. In contrast, these compounds strongly absorb lights at wavelengths between 230 and 320 nm – that is in the UV region, illustrated by their UV absorption spectra. Individual JPE could be isolated from its mixture by semi-preparative HPLC method. An isolated JPE, called *Jatropha* factor C_1 or JC1, was found to be useful as JPE standard for the optimization of LC-ESI-MS/MS instrument. By optimizing with JC1, ion pair of m/z = 293~693 was obtained as selective and intense signal. MRM analysis of JPEs with optimized m/z = 293~693 give higher selectivity and sensitivity than that with ion pair of m/z = 165~311 (optimized from PMA standard). LCMS/MS is a useful technique, not only for selective and sensitive determining for JPE but also for providing information on the chemical change of JPEs during the course of their degradation. This technique was also applied to evaluate the structural change of JC1 during methanolysis.

The degradation of JPEs and standard PMA in aqueous and methanolic solution by electrical discharge plasma at atmospheric pressure was studied (**Chapter 3**). The plasma source was dielectric barrier discharge (DBD) type and Helium was used as working gas. Sample solution was directly exposed to the plasma discharge for a specified treatment time. In aqueous solution, it was found that the degradation of JPEs was much greater than that of PMA. Complete degradation of JPEs was achieved after 15-minute-treatment. In contrast, only 16% of PMA could be degraded at the same treatment time. In case of using methanol as solvent for JPEs or PMA, complete degradation of either JPEs or PMA was observed after plasma treatment time of 15 min. Reactive oxygen species might be

responsible for the degradation of either JPEs or PMA. Furthermore, the degradation efficiency might be affected by the condition of plasma source and surrounding air. The degradation efficiency of PMA in aqueous solution was found to be improved when corona-discharge-assisted plasma, generated with argon gas, was employed. With this plasma device, larger amount of reactive species, especially hydroxy radical (•OH), could be produced according to optical emission spectrum of plasma jet and the amount of hydrogen peroxide (H_2O_2) in the treated sample.

Gamma irradiation is another method for degrading PEs. The degradation of PDA, an authentic phorbol ester, in aqueous solution by irradiation with gamma-rays from ^{60}Co radio-isotope was studied (**Chapter 4**). Aqueous PDA solution at a concentration of 10 mg/L was prepared from the methanolic PDA stock solution. The gamma-ray source with absolute dose rate of 4.56 kGy/h was used. The results show that PDA could be degraded by gamma irradiation. The degradation efficiency is increased when increasing absorbed doses. PDA was degraded to form at least one product. This product was assumed to be epoxide, according to mass spectrometric data. The degradation of PDA in aqueous solution under gamma irradiation could be induced mainly by the attack of hydroxy radical (•OH), reactive species from water radiolysis. It was found that the degradation of PDA in the presence of NO_2 (•OH radical promotor) was significantly enhanced and PDA was completely degraded at lower absorbed dose (0.5 kGy) compared with no such additive. In the presence of methanol, *tert*-butanol or 2-propanol (•OH radical scavengers), the PDA degradation was significant inhibited at absorbed dose of less than 1.5 kGy but complete degradation of PDA was still achieved at higher absorbed dose of 3 kGy. With increasing the concentration of methanol additive, the reduction of the PDA degradation efficiency at dose less than 1.5 kGy was significantly observed.

The studies on the degradation of JPEs (40 mg/L) in aqueous solution by natural sunlight (light power of 800-1180 W/m^2), simulated UV light (λ = 254 nm and power of 0.4 W/m^2) and gamma-rays (absolute dose rate of 3.78 kGy h^{-1}) were carried out (**Chapter 5**). Sample solution was transferred into a quartz cell, closed with a cap and irradiated by sunlight and UV irradiations. For gamma-ray irradiation, sample solution was carried in 400uL-glass tubes inside 2mL-vials. The irradiated sample solution was analyzed by pre-established HPLC/UV and LCMS methods. By irradiation with sunlight for 5, 10, 15, 20, 25, 30, 35 and 40 min, the JPE degradation efficiency was found to be 36, 60, 66, 82, 88, 91, 94 and 96%, respectively. The JPE degradation was found to follow pseudo-first order kinetic with a rate constant of about 1.37×10^{-3} s^{-1}. By irradiation with UV light for 5, 10, 15, 20, 25, and 30 min, the JPE degradation efficiency was found to be 67, 84, 91, 93, 97, and 99 %, respectively. The JPE degradation follows pseudo-first

order kinetic behavior with a rate constant of about 2.41×10^{-3} s^{-1}. The degradation of JPEs in aqueous solution by UV irradiation was doubly faster than by sunlight irradiation. Both intensity and wavelength of light significantly affected the direct photodegradation of JPEs in aqueous solution. By gamma irradiation, the JPEs degradation proceeded into steps. When the irradiation was conducted for 5, 10 and 15 min (0.31, 0.62 and 0.93 kGy), the JPE concentration slightly decreased which accounts for 11, 27 and 45% degradation efficiency, respectively. When the irradiation time was extended to longer for 20, 25 and 30 min (1.24, 1.55, 1.87 kGy), JPEs were gradually degraded with achieving degradation efficiency of 69, 78, and 87%, respectively. Complete degradation of JPEs in aqueous solution could be achieved by prolonging the treatment time.

In **Chapter 6**, the results of this research are summarized. Future perspectives for further studies are also included.

審査結果の要旨

　本論文は、バイオディーゼルの燃料油を得ることができる植物として近年東南アジアなどで注目されている、ジャトロファカルカスリンネ（JCL）の種子に含まれる有毒のフォルボールエステル（PE)の分解に有効と考えられる、4つの方法を適用して比較したものである。プラズマ、ガンマ線、太陽光および紫外線で PE が分解されることを確認し、またその過程を解析することで、次のような研究成果を得ている。

(1) 試料として、一般に入手できる2種の PE と JCL から抽出された PE を研究全体を通して使用した。また試料や生成物の分析および同定には、共通して質量分析と紫外線吸収スペクトル分析法を主に用いた。ヘリウムおよびアルゴンガスプラズマを照射することによってそれらが分解できることを確認した。解析の結果、分解過程に対する知見が得られ、さらに、ガスや放電の種類など、有効なプラズマの条件が明らかになった。

(2) 本学の特徴ある放射線照射施設でガンマ線照射実験を行い、生成ラジカルの作用を確認するために、ラジカルスカベンジャーを用いて分解過程を検証した。この結果、生成したヒドロキシラジカルの作用が明らかになった。

(3) 太陽光、紫外線の照射についても PE の分解が確認され、生成物の同定と分解過程の解析を行った。この結果、光照射では、光子が直接作用して PE を分解する過程が主となることが示唆された。

(4) プラズマ、ガンマ線、太陽光および紫外線で、それぞれ異なる PE の分解過程が明らかになった。このような研究はこれまでにほとんど行われておらず、バイオディーゼルの産業分野において有効な方法を確立するための重要な知見である。今後分解生成物の毒性を調べ、これらの方法を比較することが課題である。

　以上の研究成果は、化学物質の分解に可能性のある方法を適用してそれぞれの過程を明らかにしたものであり、さらに新しいエネルギー源の開発における環境問題の解決につながる可能性があり、学術・産業の発展に大きく貢献するものである。また、申請者が自立して研究活動を行うのに必要な能力と学識を有することを証したものである。学位論文審査委員会は、本論文の審査ならびに最終試験の結果から、博士（工学）の学位を授与することを適当と認める。