AAEC-LIB/TRANS-625

TRN: AU7703695

AAEC-LIB/Trans-625

DETERMINATION OF PHOSPHORUS IN CANNABIS BY NEUTRON ACTIVATION ANALYSIS - MEASUREMENT OF ^{32}P CERENKOV RADIATION BY LIQUID SCINTILLATION SPECTROMETER

by

Masaki SHINOGI and Itsuhiko MORI

Yakugaku zasshi, v.96 no.11, pp.1282-1287, 1976

Translated from the Japanese by the Japan
Information Centre of Science and Technology
May 1977

AAEC-LIB/Trans-625

AUSTRALIAN ATOMIC ENERGY COMMISSION RESEARCH ESTABLISHMENT

DETERMINATION OF PHOSPHORUS IN CANNABIS BY NEUTRON ACTIVATION ANALYSIS -MEASUREMENT OF ^{32}P CERENKOV RADIATION BY LIQUID SCINTILLATION SPECTROMETER

by

Masaki SHINOGI and Itsuhiko MORI

Yakugaku zasshi, v.96, no.11, pp.1282-1287, 1976

Translated from the Japanese by the
Japan Information Centre of Science and Technology

May 1977

AUSTRALIAN ATOMIC ENERGY COMMISSION

LIB/TRANS SERIES

DETERMINATION OF PHOSPHORUS IN CANNABIS BY NEUTRON ACTIVATION ANALYSIS -MEASUREMENT OF ^{32}P CERENKOV RADIATION BY LIQUID SCINTILLATION SPECTROMETER

by

Masaki SHINOGI and Itsuhiko MORI
Kobe Women's College of Pharmacy

The authors have already reported[3.] on the micro content elements in Cannabis by using non-destructive neutron activation analysis established by them to enable simultaneous analysis of many kinds of elements in a plant tissue. However, phosphorous cannot be analyzed by the method reported in the previous paper[3.], because no γ-ray irradiation occurs, although ^{32}P is formed as the result of thermal neutron capture reaction of phosphorous. In this paper, the results of investigation on the method of quantitative determination of phosphorous, which is an important constitutive element of a plant, by use of activation analysis. The measurement of β-rays from ^{32}P was effected by applying Cerenkov radiation measurement by using a liquid scintillation spectrometer.

^{32}P is an element formed by the $^{31}P(n,\gamma)^{32}P$ reaction due to thermal neutrons from a nuclear reactor, and has a half-life period of 14.3 days, so that the quantitative determination of phosphorous becomes possible by using the method reported in the previous paper[3.] after chemically separating phosphorous from the identical sample which has been subjected previously to simultaneous analysis of nuclear species emitting γ-rays.

As the result of recent investigations[4.] on many kinds of inorganic ion exchange agents, which are conveniently used in the separation of radioactive elements, alumina was found to adsorb phosphorous selectively, the utilization of such a result has also been investigated.

---

1. Reported in the 25th General Meeting of Japanese Pharmacy Society, Kimki Department, in Kobe, November 1975.

2. Location: Motoyama-kitamachi, Higashinada-ku, Kobe

3. Masaki Shinogi; Yasuko Murai; Itsuhiko Mori, and Takayoki Takeuchi, Journal of Pharmacy, 94, 1550, (1974).

4. F. Girardi; R. Piletra; E. Sabbioni, Journal Radioanal. Chemistry, 5, 141, (1970).

Experimental

Samples and the preparation of standard samples:

The leaves and male flowers of Cannabis at its upper and middle parts were treated by the same method as reported in the previous paper, and about 300 g of each sample were taken to weigh accurately, and were duplicatedly sealed in rinsed polyethylene sheets.

To prepare standard samples, KH_2PO_4 was weighed and dissolved into water to get a solution of 2 mg/ml in phosphorous concentration, and further, $(NH_4)_2SO_4$ and KCl were respectively weighed and dissolved in water to get solutions of 15 mg/ml in sulfur concentration and 5 mg/ml in chlorine concentration. Each 10 μl of these standard solutions was put on a milli-pore filter with diameter of 1.8 cm, and was dried and duplicatedly sealed in a polyethylene bag to be used as a standard sample.

Neutron irradiation:

Each 2 pieces of samples together with standard samples were packed in a capsule for irradiation use, and were irradiated for 1 hour in a pressurized air transfer tube No.2 (thermal neutron flux 2.75×10^{13} neutron/cm^2/sec, fast neutron flux 6.0×10^{12} neutron/cm^2/sec) in the Kyoto University Nuclear Reactor Experimental Station.

Chemical separation of phosphorous:

Samples and standard samples were cooled for 2 weeks after irradiation to let short-life nuclear species decay, and then, were decomposed in a wet manner with concentrated nitric acid and 70% perchloric acid in a Bethge decomposition apparatus[6]. The decomposed liquids were evaporated on a water bath to dryness, and the solids obtained were dissolved in 1M perchloric acid to be passed through a polyethylene column (8 mm in internal diameter and 20 cm in length) filled with 3 g of aluminium oxide to make phosphorous be adhered on alumina. Further, after washing the column with 1M perchloric acid, phosphorous was eluted with 1M hydrogen fluoride solution. The effluent was partitioned into each 12 ml portion in a polyethylene vial.

Measurement of Cerenkov radiation of ^{32}P:

To the phosphorous eluate separated from the sample was added 4-methyl umbelliferron (200 mg/l) as a wave length shifter, and the Cerenkov radiation of ^{32}P was measured by means of a liquid scintillation spectrometer (GSL-263 type, made by Fujitsu Co., Ltd.). The measured values were corrected by use of a quenching correction curve.

---

5. E. Sabbioni; R. Pietra; F. Girardi, Journal Radioanal. Chemistry, 4, 289, (1970).

6. P.O. Bethge, Anal. Chemistry, 28, 119, (1956).

On the other hand, each one of the middle part leaves and standard samples were put in a polyethylene vial as they are contained in the inner bag after peeling off the polyethylene outer bag to carry out measurement by the dry counting method.

Further, measurement was continued for 49 days after irradiation, and the decay corresponding to the half-life of ^{32}P was confirmed.

Quantitative determination of phosphorous by the molybdenum blue method:

To compare with the results of activation analysis, the generally well known quantitative determination method of phosphorous, i.e. the molybdenum blue method, was applied to the phosphorous determination in Cannabis.

About 200 mg of the sample was heated in an electric furnace at 450°C to convert into dry ash, and a small amount of concentrated hydrochloric acid and water was added to the ash. The ash solution was then evaporated on a water bath to dryness. The dried product was added with 2 ml of 1:3 hydrochloric acid and 10 ml of water, and the mixture was heated on a water bath and then was filtered. The residue was washed well, and put into a 100 ml measuring flask together with the filtrate, and further added with water up to 100 ml. Two milli-litre of this diluted solution was taken in a 25 ml measuring flask, and after adding 2 ml of ammonium molybdate solution, 2 ml of 0.5% hydroquinone solution, and 2 ml of 10% sodium sulfite solution, water was further added up to 25 ml. After 30 minutes, absorptivity was measured at the wave length of 650 nm.

Results

After 14 days after irradiation, ^{32}P was chemically separated and Cerenkov radiation was measured. The decay curves measured are shown in Fig. 1.

All samples showed the half-life decay of ^{32}P, and after observing the radioactivity just after irradiation, the phosphorous content in each sample was obtained by the comparison method with standard samples. The results are shown in Table 1 in comparison to those of the molybdenum blue method.

From the above-described results, the phosphorous content is in the order as middle plant leaves < upper part leaves < male flowers. The result showed the importance of phosphorous as a constitutional element just as pointed out hitherto.

Consideration

Investigation in the chemical separation of phosphorous:

In the separation of ^{32}P, an inorganic ion-exchange was used, which is stronger against irradiation and more heat-resistant than the ion-exchange resins hitherto utilized. As adsorbents for phosphorous, there are such materials as (1) alumina, (2) tin dioxide, (3) manganese dioxide, (4) zirconium phosphate, etc., but according to the papers reported by Girardi et al.[4.], acidic alumina (referred to hereafter as AAO) shows the best selectivity for phosphorous, so that alumina was used to examine the adsorptive ability of phosphorous.

As the acid solutions, in which phosphorous is adsorbed on AAO, there are nitric acid and perchloric acid, but, as the latter has the greater adsorptive power[5.], and as nitric acid causes quenching in the measurement of Cerenkov radiation, perchloric acid was used to measure the adsorptive power for phosphorous in various acid concentrations. The results are shown in Fig. 2.

As a result, no difference in adsorptive power was caused by the change of perchloric acid concentration, so that 1M perchloric acid (1.16 mgP/g. AAO) was adopted for use.

Next, 300 mg of a cannabis leaf was wet decomposed, and a definite amount of ^{32}P was added to the decomposed solution as a tracer. Then, the adsorptive power for phosphorous in relation to the amount of AAO was studied. The results are shown in Fig. 3.

The adsorption rate of AAO was 99.6% for 2.5 g and 99.8% for 3.0 g, so that 3.0 g AAO was adopted to use.

On the other hand, 1M hydrofluoric acid was used to elute the phosphorous adsorbed on AAO, and the elutability was found to be 98.8%, when elution was effected twice on 12 ml.

Therefore, the recovery percentage in chemical separation of phosphorous, by taking adsorption rate and elutability into consideration, became 98.6%. As standard samples were also chemically treated in the same manner, errors in the recovery percentage could be neglected.

Investigation in Cerenkov radiation measuring conditions for ^{32}P:

The advantages of Cerenkov radiation measurement reside in that it requires no organic solvent, and that it can be applied to the sample water solutions as they are, and further, that its cost is low because no scintillator (PPO, etc.) is necessary.

Also, its counting efficiency is difficultly influenced by high concentration solutions of salts other than the NO_2 salt, chemical light extinguishers, acids and alkalides, and therefore, the accuracy of measurement increases when the amount of samples is abundant. As the β-ray energy of ^{32}P is higher than the critical energy 0.263 MeV required for irradiating Cerenkov radiation in an aqueous solution, the measurement conditions for Cerenkov radiation by using a liquid scintillation spectrometer was investigated.

(1) On the amount of solutions measured

In vials for measuring use, 1 ml to 15 ml of water was put, and in each vial was added a definite amount of ^{32}P to measure counting rate. As shown in Fig. 4, almost no difference was found when the liquid volume changed from 11 ml to 15 ml, the liquid volume in measurement was determined as 12 ml.

(2) On the effect of acids

As to the nitric acid, perchloric acid, and hydrofluoric acid, their effects on the counting rate in various concentrations of respective acids were measured.

The results are shown in Fig. 5.

In the cases of perchloric acid and hydrofluoric acid, almost no effect of the acid concentration to the counting rate was observed, but in the case of nitric acid, marked decrease in counting rate was shown, when the concentration had increased. From this fact, it can be conjectured that nitric acid can become a cause for large quenching in the measurement of Cerenkov radiation.

(3) Correction for quenching

In Cerenkov radiation measurement, the most significant problem is, beside the quenching due to nitric acid, the liability to the effect of colour quenching.

It is difficult to make the sample perfectly colourless by wet decomposition, and in many cases, a little yellow colour remains, and moreover, the colour depth is not constant. Therefore, a correction curve for correcting such quenching was prepared. That is, sample decomposition liquids in various states were prepared, to which was added a known amount of ^{32}P. Then, the counting efficiency was measured. The result was plotted in relation to the channel ratio in the same case to obtain the correction curve as shown in Fig. 6.

This curve was used in correcting the quenching of measured valves.

(4) On the dry counting method

The dry counting method was studied, in which ^{32}P was adsorbed on AAO and put as it was into a polyethylene vial, taking the loss in chemical separation and the effect of colour quenching into consideration.

The amount of AAO was varied in measurements against the definite amount of ^{32}P.

As the amount of AAO increased, the self absorption by AAO showed a greater effect, and the counting rate reduced. To separate phosphorous from the sample, 3.0 g of AAO was used in the measurement, so that the determination by use of the dry counting method was difficult in this method, becasue the counting rate was very bad and the counting rate changes with the amount of AAO.

Further, comparison of the results by use of the dry counting method for quantitative determination in which the powder of cannabis leaves after neutron irradiation as it was, was put in a polyethylene vial, with the results after effecting chemical separation showed that the former amounted only 54.8% of the latter. This can be considered obviously due to the self absorption and directional properties of the sample.

(5) On the interfering nuclear species

In the Cerenkov radiation measurement of ^{32}P, the main ones of interfering nuclear species are, according to the report by Sabbioni et al., ^{198}Au, ^{60}Co, ^{134}Cs, ^{192}Ir, ^{191}Os, ^{86}Rb, ^{136}Re, $^{122+124}Sb$, ^{46}Sc, and ^{182}Ta. On the other hand, elements, which are adsorbed by AAO in an 1M perchloric acid solution, are Ge, As, Nb, Mo, Sb, Ta, W, Os, Au, Pa, etc. As the nuclear species which are common to both series are ^{198}Au, ^{191}Os, ^{182}Ta, and $^{122+124}Sb$, which respectively give 1.45, 0.05, 0.037, and 0.15 fold counting rates against the counting rate of ^{32}P. However, above-described 4 elements are markedly small in the existence ratio in a plant body, and moreover, the adsorbability to AAO is low in them except Sb, so that they can be considered not to interfere in the case when Cerenkov radiation is measured after chemically separating ^{32}P. Furthermore, after the irradiated sample had been passed through the AAO column, the column was subjected to γ-ray spectrometry by means of a Ge(Li) detector, but no prominent peak was detected. From this fact also, the effect of the coexistence of interfering nuclear species can be considered not to be large.

However, when the sample was irradiated in a nuclear reactor, other than $^{31}P(n,\gamma)^{32}P$, $^{32}S(n,p)^{32}P$ and $^{35}Cl(n,\alpha)^{32}P$ reactions will be formed by fast neutrons, to form the same nuclear species ^{32}P. As it is impossible to prevent these interfering reactions, it was experimentally investigated that in what ratio the formation would occur, and the decay curve shown in Fig. 8 could be obtained.

The standard samples were put into polyethylene vials as they are contained in milli-pore filters, and after irradiation, measurement for them was repeated after 1 to 49 days.

As a potassium salt was used as a standard sample, a high counting rate was shown due to the effect of ^{42}K (T = 12.5 h) until 1 week after its radioactivity decayed, but fron 10 to 49 days, decay was observed to follow the half-life of ^{32}P. When the decay curves of ^{32}P formed from phosphorous, sulfur and chlorine were extended, and from the counting rate just after irradiation, the ^{32}P contributions from sulfur and chlorine to phosphorous were calculated. If all elements were assumed to exist in the same amount, the contribution of sulfur to phosphorous was 6.8% and that of chlorine to phosphorous was 1.9%. However, when the amount of existence of these 3 elements in a plant body (P:S:Cl = 1:0.71:0.29) according to the values in a bibliography[8.] was taken into consideration, the error based on sulphur and chlorine becomes about 5%, and can be included in the error range of usual activation analyses.

Conclusion

The activation analysis utilizing Cerenkov radiation measurement in analyzing phosphorous in living tissue samples can be sufficiently used.

In the chemical separation of phosphorous, the use of AAO makes the separation of ^{32}P simple and convenient, and also effective in removing interfering nuclear species in the Cerenkov radiation measurement.

When the limit of detection of the counting rate is so assumed that it reaches to the two folds of that of the background, the detection limit in this method will be determined as 0.4 ppm for the 300 mg sample, as it reaches to 0.13 μg when treatment and measurement of the sample were effected after 40 days of irradiation. However, when treatment is effected until 1 week after irradiation, the detection limit can be avoided until up to 0.026 μg. Therefore, the present method has a better detection sensitivity than that of conventional calorimetric methods.

---

7. E. Sabbioni; L. Clerici; F. Girardi; F. Campagnari, Journal Radioanal. Chemistry, 14, 159, (1973).

Acknowledgement

The authors are very grateful to both students, Miss Giyoko Oyama and Miss Kayoks Ishida, who have co-operated in this study.

---

8. "Fundamental Lectures on the Marine Science, vol.11, Marine Biology", Edited by Akihiko Hattori, Tokai University Publishing Society, Tokyo, 1973.

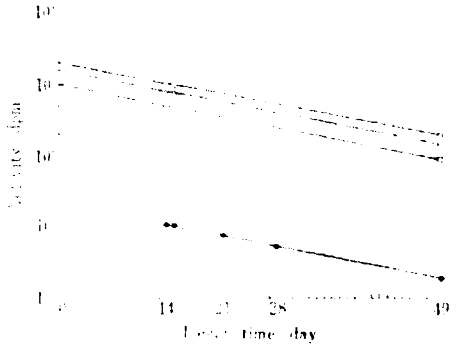

Fig. 1. Decay Curve of Cerenkov Radiation after Chemical Separation of Phosphorus

neutron flux [illegible] n/cm² sec; irradiation [illegible]

[illegible] leaves

upper part leaves

[illegible] part leaves

● phosphorus

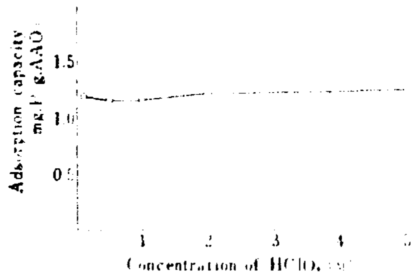

Fig. 2. Adsorption Capacity for Phosphorus on Acid Aluminum Oxide from Various Molarity of $HClO_4$ Media

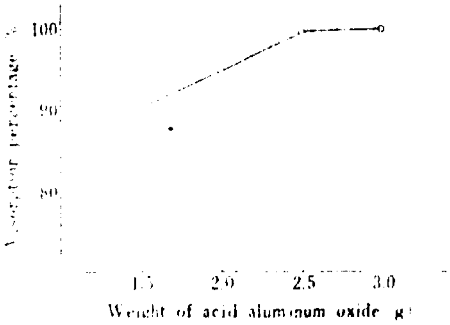

Fig. 3. The Relationship between Adsorption Percentage and Weight of Acid Aluminum Oxide

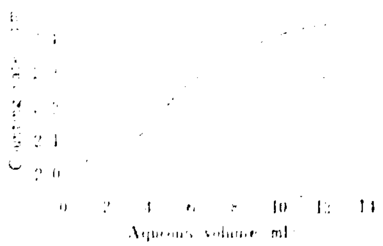

Fig. 4. The Influence of Sample Volume in Polyethylene Vial on Cerenkov Radiation Counting

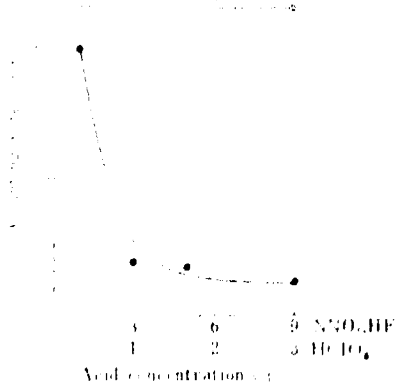

Fig. 5. The Influence of Various Molarities of HNO_3, HF and $HClO_4$ on Cerenkov Radiation Counting

$HClO_4$ HF ● HNO_3

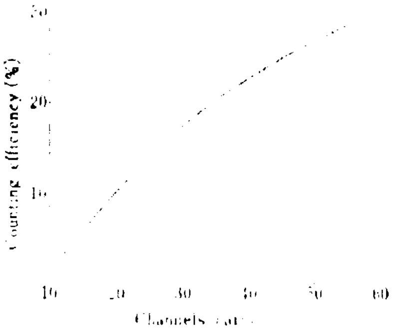

Fig. 6. Cerenkov Ratio Quench Correction Curve for ^{32}P in Water

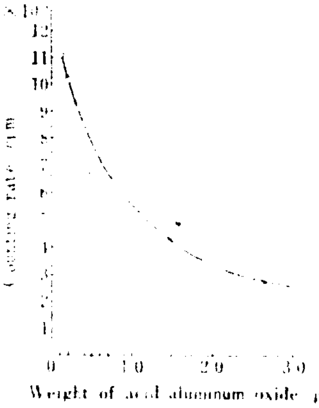

Fig. 7. The Influence of Weight of Acid Aluminum Oxide on Cerenkov Radiation Dry Counting

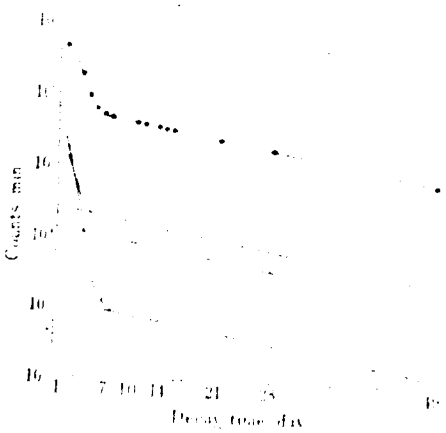

Fig. 8. The Influence of Coexisting Sulfur and Chlorine to the Determination of Phosphorus

Neutron flux, thermal 2.7 × 10¹² n/cm²·sec [illegible]
[illegible] irradiation
● Cannabis part I [illegible]
sulfur [illegible] mg
phosphorus 29 μg
chlorine [illegible] mg

Table I. Concentration of Phosphorus in Cannabis

Cannabis	Activation analysis	Molybdenum blue method
Male flower	[illegible]	0.70%
Upper part leaves	[illegible]	0.58%
Middle part leaves	[illegible]	0.29%

52-99

YAKUGAKU ZASSHI
96 (11) 1282-1287 (1976)

UDC 546.18.02.32.09: 541.28.08

中性子放射化分析法による大麻中のリンの定量 —^{32}P の液体シンチレーションスペクトロメーターによるチェレンコフ光測定[1]—

志野木正樹，森　五彦
神戸女子薬科大学[2]

Determination of Phosphorus in Cannabis by Neutron Activation Analysis —Measurement of ^{32}P Cerenkov Radiation by Liquid Scintillation Spectrometer[1]—

Masaki Shinogi and Itsuhiko Mori
Kobe Women's College of Pharmacy[2]

(Received January 14, 1976)

Thermal neutron activation analysis with measurement of ^{32}P Cerenkov radiation by liquid scintillation spectrometer was used to determine phosphorus in cannabis. After irradiation of the sample, wet ashing was carried out with conc. nitric acid and 70% perchloric acid. The solution in 1 M perchloric acid transferred to an inorganic ion-exchange column containing acid aluminum oxide and phosphorus was quantitatively eluted with 1 M hydrofluoric acid. The ^{32}P radioactivity of each fraction of the eluate was counted with Cerenkov radiation by a liquid scintillation spectrometer from 2 to 7 weeks after the irradiation. The activity curve decayed with ^{32}P half life. The isotope channel ratio technique was applied for the quench correction.

The values for upper and middle leaves, and male flower of cannabis, in parts per million, were 0.55, 0.31, and 0.71, respectively, and a good agreement was found with the values from Molybdenum Blue method. The determination limit of phosphorus by this method was calculated to be 2.5×10^{-8} g.

The optimal experimental conditions for chemical separation of phosphorus and for measuring the ^{32}P Cerenkov radiation were also examined.

著者らは，すでに植物体中の多種類元素を同時に分析する非破壊中性子放射化分析法を確立し，大麻試料中の微量元素について報告した.[3] しかし，リンは熱中性子捕獲反応により ^{32}P を生成するが，γ線を放出しないので前報[3] の方法により分析することはできない．本報では，植物の生元素として重要なリンを，放射化分析により定量する分析方法を検討したので報告する．なお，^{32}P の β線測定には，液体シンチレーションスペクトロメーターを用いるチェレンコフ光測定を応用した.

^{32}P は原子炉の熱中性子で ^{31}P$(n,\gamma)^{32}$P 反応によって生成し，半減期が 14.3 日であるので，前報[3] の方法により，γ線を放出する核種の一斉分析を行なった後，同一試料から化学的に分離しリンを定量することが可能である.

また，最近放射性元素の分離に都合のよい種々の無機イオン交換体が研究されており,[4] アルミナがリンを選択的に吸着することも知られた[5] ので，その利用についても検討した.

実験

試料および標準試料の調製　試料として大麻の上部および中部の葉と雄花を前報[3] の方法にて処理したものを約 300 mg ずつ精秤し，洗浄したポリエチレン袋に 2 重に封入した.

---

[1] 日本薬学会近畿支部第 25 回総会で発表，神戸，1975 年 11 月.
[2] Location: *Motoyama-kitamachi, Higashinada-ku, Kobe.*
[3] 志野木正樹，村井康子，森　五彦，武内孝之，薬誌，**94**, 1550 (1974).
[4] F. Girardi, R. Piletra, E. Sabbioni, *J. Radional. Chem.*, **5**, 141 (1970).
[5] E. Sabbioni, R. Pietra, F. Girardi, *J. Radioanal. Chem.*, **4**, 289 (1970).

標準試料として KH_2PO_4 をリン濃度として 2 mg/ml になるように秤量し，水に溶解した．さらにイオウとして 15 mg/ml，塩素として 5 mg/ml になるように $(NH_4)_2SO_4$ および KCl をそれぞれ秤量し，水に溶解した．これらの標準溶液を，それぞれ直径 1.8 cm のミリポアフィルター上に 10 μl ずつ付けて乾燥させ，試料と同様にポリエチレン袋に2重に封入し，標準試料とした．

中性子照射　試料を標準試料と共に各々2個づつ照射用カプセルにつめ，京都大学原子炉実験所の圧気輸送管 No. 2 (熱中性子束 2.75×10^{13} neutron/cm²/sec，速中性子束 6.0×10^{12} neutron/cm²/sec) において1時間照射した．

リンの化学分離　試料および標準試料を，照射後2週間冷却し，短寿命核種の減衰を待って，Bethge の分解装置[6] を用いて濃硝酸および 70% 過塩素酸で湿式分解し，分解液を水浴上で蒸発乾固させ，1M 過塩素酸に溶解させて，3g の酸化アルミナを充塡したポリエチレンカラム（内径 8 mm，長さ 20 cm）に通し，リンをアルミナに吸着させさらに 1M 過塩素酸で洗浄した後，1M フッ化水素酸でリンを溶出させた．流出液を 12 ml づつポリエチレン製のバイアルに分取した．

^{32}P のチェレンコフ光測定　試料より分離したリンの溶出液に波長シフターとして 4-メチルウンベリフェロン (200 mg/l) を加え，液体シンチレーションスペクトロメーター（富士通 K.K. 製 GSL-263 型）により，^{32}P のチェレンコフ光を測定した．測定値はクエンチング補正曲線を用いて補正を行なった．

一方，中部の葉および標準試料の各々1つをポリエチレンの外袋をはがし，内袋のままポリエチレン製バイアルに入れ，dry counting 法により測定した．

なお，照射後 49 日まで測定し，^{32}P の半減期に従って減衰していることを確めた．

モリブデン青法によるリンの定量　放射化分析の結果と比較するために一般的なリンの定量法であるモリブデン青法による大麻試料中のリンの定量を行なった．

試料約 200 mg を 450° の電気炉中で乾式灰化し，少量の濃塩酸と水を加えて水浴上で蒸発乾固させ，1:3 の塩酸 2 ml と水 10 ml を加えて水浴上で加熱してろ過し，残査をよく洗浄してろ液と共に 100 ml のメスフラスコにとり，水で 100 ml とした．この希釈液 2 ml を 25 ml のメスフラスコにとり，モリブデン酸アンモン溶液 2 ml，0.5% ヒドロキノン液 2 ml，10% 亜硫酸ナトリウム溶液 2 ml を加え，水で 25 ml とした．30 分後に波長 650 nm で吸光度を測定した．

結　　果

照射後 14 日後に ^{32}P を化学分離し，チェレンコフ光を測定した試料の減衰曲線を Fig. 1 に示す．

各試料とも ^{32}P の半減期で減衰しており，照射直後の放射能を求め，標準試料との比較法により各試料中のリン含有量を求めた．その結果を，モリブデン青法の結果と比較して Table 1 に示した．

以上より，リン含有量は，中部の葉＜上部の葉＜雄花の順に多く含有されており，従来より指摘されているように，生元素としてのリンの重要性を示す結果を得た．

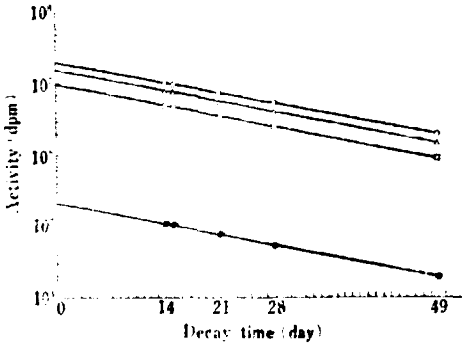

Fig. 1. Decay Curve of Cerenkov Radiation after Chemical Separation of Phosphorus

(neutron flux; thermal 2.75×10^{13} n/cm²/sec.; irradiation time; 1 hour)

—○—: male flower　　—△—: upper part leaves
—□—: middle part leaves　　—●—: phosphorus

TABLE I. Concentrations of Phosphorus in Cannabis

Cannabis	Activation analysis	Molybdenum blue method
Male flower	0.71%	0.70%
Upper part leaves	0.55%	0.58%
Middle part leaves	0.31%	0.29%

---

6) P.O. Bethge, *Anal. Chem.*, **28**, 119 (1956).

考　　　察

リンの化学分離の検討

^{32}P の分離に対し，従来より利用されていたイオン交換樹脂よりも放射線に強く，耐熱性の良い無機イオン交換体を用いた．リンを吸着するものとしては，1) アルミナ，2) 二酸化スズ，3) 二酸化マンガン，4) リン酸ジルコニウムなどがあるが，Girardi らの文献[9] より，リンに対して最も選択性の良い酸性アルミナ（以後 AAO と略す）を使用し，リンの吸着能を検討した．

AAO にリンを吸着させる酸溶液としては，硝酸と過塩素酸があるが，後者の方が吸着能力が大きい[9] こと，および硝酸はチェレンコフ光測定時においてクェンチングの原因になることから過塩素酸を用いることにし，種々の濃度におけるリンの吸着能力を検討し Fig. 2 に示した．

この結果，過塩素酸濃度による吸着力の差はほとんどみられなかったので，1M 過塩素酸を (1.16 mgP/g. AAO) 使用することにした．

次に試料の大麻葉 300 mg を湿式分解し，その分解液に一定量の ^{32}P をトレーサーとして加え，AAO の量によるリンの吸着能力を検討し Fig. 3 に示した．

AAO の量が 2.5 g の時 99.6%，3.0 g で 99.8% の吸着率であったので AAO 3.0 g を使用することにした．

一方，AAO に吸着させたリンを溶出させるために 1M フッ化水素酸を用いたが，12 ml ずつ 2 回溶出させた時の溶出率は 98.8% であった．

ゆえに，吸着率および溶出率を考慮したリンの化学分離における回収率は 98.6% であった．なお，標準試料も同様の化学処理を行なうため，この回収率の誤差は無視することができる．

^{32}P のチェレンコフ光測定条件の検討

チェレンコフ光測定の長所は，有機溶媒を用いず，水溶液のままで測定でき，シンチレーター (PPO など) が不要なので廉価である．

また，NO_2 塩以外の高濃度の塩溶液，化学消光剤，酸やアルカリなどで計数効率は影響されにくく，したがって試料の量が多いときには測定の正確度が増加する．なお，^{32}P の β 線エネルギーは水溶液中でチェレンコフ光を放射させるのに必要な臨界エネルギー 0.263 MeV より高いため，^{32}P の β 線測定に液体シンチレーションスペクトロメーターを用いるチェレンコフ光測定条件を検討した．

1) 測定溶液量について　測定用バイアルに水 1 ml から 15 ml まで入れ各々のバイアルに一定量の ^{32}P を加えて計数率を測定した．Fig. 4 より，11 ml から 15 ml まではとんど差がなかったので，測定時の液量を 12 ml とした．

2) 酸の影響について　試料分解から，リンの分離までに用いる，硝酸，過塩素酸，フッ化水素酸について，それぞれ種々の濃度における計数率への影響を検討し，Fig. 5 に示した．

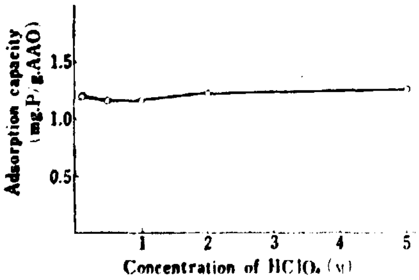

Fig. 2. Adsorption Capacity for Phosphorus on Acid Aluminum Oxide from Various Molarity of $HClO_4$ Media

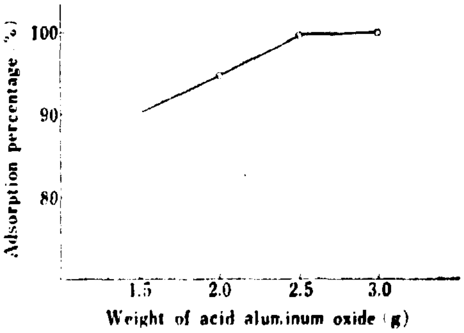

Fig. 3. The Relationship between Adsorption Percentage and Weight of Acid Aluminum Oxide

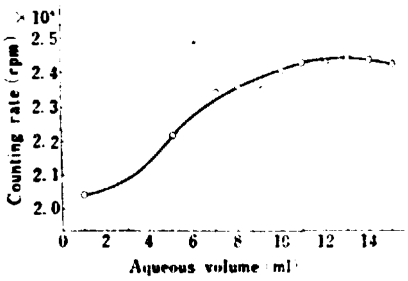

Fig. 4. The Influence of Sample Volume in Polyethylend Vials on Cerenkov Radiation Counting

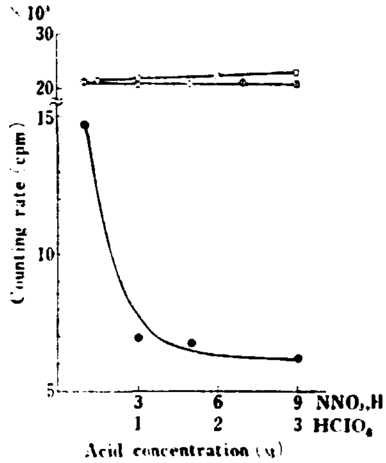

Fig. 5. The Influence of Various Molarities of HNO_3, HF and $HClO_4$ on Cerenkov Radiation Counting

—○—: $HClO_4$　—◎—: HF　—●—: HNO_3

過塩素酸およびフッ化水素酸については酸濃度による計数率への影響はほとんどないが，硝酸については，濃度が増加すると著しい計数率の減少を示す．このことにより硝酸はチェレンコフ光測定時において大きなクェンチングの原因になることが推定できる．

3) クェンチングの補正　チェレンコフ光測定においては，硝酸によるクェンチングと共に，一番問題になるのはカラークェンチングの影響を受けやすいことである．

試料を湿式分解にて完全に無色にすることは困難で，多少黄色に着色する場合が多く，しかも，その色濃度は一定にはならない．ゆえにこれらのクェンチングを補正する補正曲線を作成した．すなわち，種々の状態の試料分解液を作り，これに既知量の ^{32}P を加えて測定し，計数効率を求め，その時の Channel Ratio との関係を目盛り，Fig. 6 の補正曲線を得た．

この曲線を用いて測定値のクェンチングを補正した．

4) Dry Counting 法について　化学分離における損失や，カラークェンチングなどの影響を考慮して，^{32}P を AAO に吸着させ，そのままポリエチレンバイアルに入れて測定する dry counting 法について検討した．

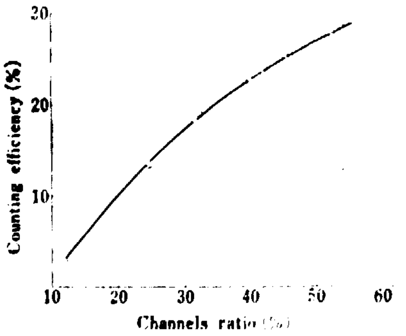

Fig. 6. Channels Ratio Quench Correction Curve for ^{32}P in Water

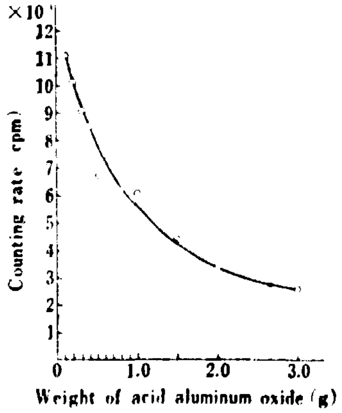

Fig. 7. The Influence of Weight of Acid Aluminum Oxide on Cerenkov Radiation Dry Counting

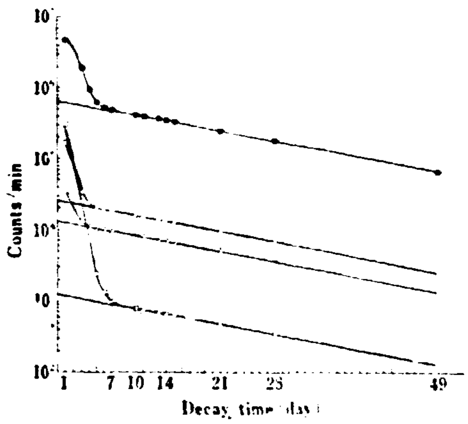

Fig. 8. The Influence of Coexisting Sulfur and Chlorine to the Determination of Phosphorus

(neutron flux; thermal 2.75×10^{12} n/cm²/sec.; fast 6.0×10^{10} n/cm²/sec., 1 hour irradiation)
—●—: middle part leaves　—○—: phosphorus 20 μg
—△—: sulfur 150 μg　—|—: chlorine 50 μg

一定量の ^{32}P に対し AAO の量をかえて測定し Fig. 7 の結果を得た.

AAO の量が増加すれば，AAO による自己吸収が大きく影響し，計数率の減少が認められる．試料より，リンを分離するためには 3.0 g の AAO を用いるため，この方法では計数率が非常に悪いことと，AAO の量によって計数率がかわることより dry counting 法を用いた定量は困難である．

さらに大麻の中葉について化学分離を行なわずに，中性子照射したままの大麻葉の粉末をポリエチレン製バイアルに入れ，dry counting 法で定量した結果と化学分離を行なった結果を比較してみると，前者は後者の 54.8% しかなかった．あきらかに自己吸収および試料の方向性のためと思われる．

5) 妨害核種について　^{32}P のチェレンコフ光測定時において，妨害となる核種は Sabbioni らの報告[7] によると，その主なものは ^{198}Au, ^{60}Co, ^{134}Cs, ^{192}Ir, ^{191}Os, ^{86}Rb, ^{186}Re, $^{122+124}$Sb, ^{46}Sc, ^{182}Ta である．一方，1M 過塩素酸溶液で AAO に吸着する元素としては Ge, As, Nb, Mo, Sb, Ta, W, Os, Au, Pa などがあり，どちらにも共通している核種としては，^{198}Au, ^{191}Os, ^{182}Ta, $^{122+124}$Sb があり，それぞれ ^{32}P の計数率に対して 1.45, 0.05, 0.037, 0.15 倍の計数率を与える．しかし，植物体中の存在比より上記4元素はリンに対して著しく少なく，さらに AAO に対する吸着率も Sb を除き低いことより，^{32}P を化学分離し，チェレンコフ光を測定する場合には妨害となることはないものと思われる．なお，照射した試料を AAO カラムに通した時のカラムを Ge(Li) 検出器で γ 線スペクトロメトリーを行なったが顕著なピークは検出されなかった．このことからも妨害核種の共存による影響は大きくないと考えられる．

しかし，試料を原子炉で照射した場合，^{31}P(n,γ)^{32}P 以外に，速中性子によって ^{32}S(n,p)^{32}P および ^{35}Cl(n,α)^{32}P 反応がおこり，同じ核種 ^{32}P が生成する．これらの妨害反応を防ぐことは不可能なため，どのような割合で生成するかを実験的に検討し，Fig. 8 のような減衰曲線を得た．

標準試料はミリポアフィルターのままポリエチレンバイアルに入れて照射後1日後から49日後まで測定した．

標準試料としてカリウム塩を使用したので ^{42}K(T = 12.5 h) の影響により，その放射能が減衰する1週間後までは高い計数率を示しているが，10日から49日までは ^{32}P の半減期で減衰している．リン，イオウ，塩素より生じる ^{32}P の減衰曲線を延長し，照射直後の計数率からリンに対するイオウおよび塩素からの ^{32}P 寄与を計算した．もしすべて同量存在するとすれば，リンに対するイオウは 6.8%，塩素は 1.9% の寄与であった．しかし，文献値[8] より植物体中のこれら3元素の存在量 (P : S : Cl = 1 : 0.71 : 0.29) を考慮すれば，イオウ，塩素に基因する誤差は 5% 程度となり，通常の放射化分析の誤差範囲に収まる．

結　　語

生体試料中のリンの分析にチェレンコフ光測定を利用した放射化分析法は十分使用することができる．また，リンの化学分離には，AAO の使用は，^{32}P の分離を簡便にし，さらにチェレンコフ光測定における妨害核種の除去にも有効である．

---

7) E. Sabbioni, L. Clerici, F. Girardi, F. Campagnari, *J. Radioanal. Chem.*, 14. 159 (1973).
8) 服部明彦編，"海洋科学基礎講座 11巻 海洋生化学，" 東海大学出版会，東京，1973.

なお，計数率がバックグラウンドの 2 倍の時を検出限界とすれば，照射後 40 日後に試料を処理し測定した時，0.13 μg となり，300 mg の試料では 0.4 ppm がこの方法における検出限界とされる．しかし，照射後 1 週間までに処理すれば 0.026 μg まで検出限界はさげられる．したがって本法は従来の比色法などよりも検出感度は良好である．

謝辞　本研究に協力いただいた 大山喜代子，石田加代子 両学生に深謝します．