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CARBONIZATION OF WOOD BY DEHYDRATING AGENT

Part III*

On the Adsorption of Penicillin on Hydrated
Active Charcoal (H-A Charcoal) and its
Elution with Aqueous Acetone

By

Michio HANZAWA and Seiichi SATONAKA**

脱水剤による木材炭化 (第III報)

水和活性炭のペニシリンの吸着およびアセトン
水溶液による脱着について

半 沢 道 郎

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In the first period of penicillin manufacture, a process has been used that comprised adsorption of crude penicillin on the active carbon from its culture fluid, elution with aqueous acetone, followed by concentration and further purification.

In order to investigate the type of adsorption and elution of penicillin on the hydrated active charcoal that was made from wood, and whether this charcoal can be put to practical use, some studies were made with the H-A charcoal which had been prepared in our laboratory. The experiments were carried out at the factory of Sapporo Pharmaceutical Plant of Hokkaido Rakunō Kyodō Kabushiki Kaisha (Hokkaido Dairy Farming Cooperative Co. Ltd.) at that time.

Lately a progress has been made in penicillin manufacture, and the adsorption method is not adopted usually, however these experimental data are interesting to show the properties of H-A charcoal.

* Part II is published in this bulletin.

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(1) Method of experiment

A certain quantity of crude penicillin culture fluid or aqueous solution of purified penicillin was added to a certain amount of H-A charcoal. The mixture was shaken for 10 minutes on a shaking apparatus (amplitude: ca. 10 cm, 120 times per min.), then filtered with Büchner's funnel under suction and the filtrate was taken in a test-tube. The charcoal that adsorbed penicillin was transferred into aqueous acetone (ca. 80 per cent by weight) together with the filter paper, and after being shaken for 10 minutes this was filtered and the filtrate was transferred into a test-tube. Both filtrates were assayed by the official method described in the next paragraph.

(2) The method of assaying penicillin

The quality of penicillin is usually indicated by the potency of its biological activities¹⁾, and one unit is the least amount of penicillin to inhibit the growth of *Staphylococcus aureus* in 50 mℓ of bouillon, or one unit contains 0.0006 mg of pure crystal of sodium salt of penicillin II (penicillin G).

There are various methods for assaying penicillin, however in this experiment, the filter paper method²⁾ was adopted, because the treatments and calculation are easy and convenient. By this method many assays can be run simultaneously to test a several Petri-dishes.

On the surface of agar which was coagulated in the bottom of the dish was poured the culture liquid of staphylococcus. The solution for assay was diluted to 4-49 units of penicillin in one mℓ. A sterilized small circular filter paper (6.5 mm in diameter) was wetted moderately with this diluted solution with a sterilized pincette, and this filter paper was placed on the agar surface in the Petri-dish. Five to seven papers may be placed in one Petri-dish. The Petri-dishes were then transferred into the incubator at 38° for 16-18 hours. The diameters of circles which were made transparent by the inhibiting power of penicillin were measured by a scale. The potencies of the test solutions were determined by the figures which had been prepared previously with standard penicillin that are given in Table 1.

In this experiment the potency of penicillin is that of content in one mℓ in every case.

(3) Adsorption and elution of penicillin on H-A charcoal

Experiment A. H-A charcoal made from most common woods in Hokkaido and from decayed wood.

Table 1. Relation between the potency of penicillin and the diameter of the circle of inhibition

| Diameter (mm) | Potency (unit) | Diameter (mm) | Potency (unit) |
|------------------|-------------------|------------------|-------------------|
| 9.0 | 4.0 | 16.5 | 16.0 |
| 9.5 | 4.3 | 17.0 | 17.5 |
| 10.0 | 4.7 | 17.5 | 18.5 |
| 10.5 | 5.0 | 18.0 | 20.0 |
| 11.0 | 5.4 | 18.5 | 23.5 |
| 11.5 | 5.8 | 19.0 | 27.0 |
| 12.0 | 6.2 | 19.5 | 30.0 |
| 12.5 | 6.8 | 20.0 | 32.5 |
| 13.0 | 7.3 | 20.5 | 35.0 |
| 13.5 | 7.8 | 21.0 | 37.5 |
| 14.0 | 9.0 | 21.5 | 40.0 |
| 14.5 | 10.0 | 22.0 | 42.5 |
| 15.0 | 11.5 | 22.5 | 45.0 |
| 15.5 | 13.0 | 23.0 | 49.0 |
| 16.0 | 14.5 | | |

The hydrated active charcoals which were described in the previous report³⁾ (Experiment No. (I) 1, (I) 2, (I) 3, (I) 4, (II) 1, (II) 2, (II) 3, (II) 4, (III) 1, (III) 2, (III) 3, and (III) 4) were tested for the adsorption and elution of penicillin by the following procedure. Each 0.3 g equivalent weight* of the charcoal was placed in a ca. 100 mℓ flask fitted with a glass stopper, and 15 mℓ of crude culture fluid of penicillin was added. The quantity of the charcoal was about 2 per cent that of the fluid. The penicillin was adsorbed in the above mentioned way and was eluted with 10 mℓ of ca. 80 per cent (by weight) aqueous acetone. Potency of penicillin in the filtrate (crude culture solution of which penicillin was adsorbed by the charcoal) and the aqueous acetone solution were assayed by the above described method.

The results are given in Table 2 and Fig. 1.

Experiment B. Samples (H-A charcoal) which had been prepared previously.

* The charcoals were taken with an equivalent weight of oven dry weight, calculated from the percentage of moisture content, because these charcoals had been stored in wet state.

Table 2. Adsorption and elution of penicillin by H-A charcoals from the most common woods in Hokkaido

| Exp. A. ○* | | | | | |
|--------------------------------|----------------|-------------------|---------------|-------------------|--------------------------|
| Crude penicillin culture fluid | | pH | Potency | Average | |
| at beginning | | 7.6 | 100.0 unit | 82.5 unit | |
| after experiment | | 7.6 | 65.0 | | |
| Charcoal Exp. No. | P. in filtrate | | P. in acetone | | Note |
| | pH | Potency (unit) | pH | Potency (unit) | |
| (I) 1 | 5.4 | 11.5 | 5.4 | 54.0 | "todomatsu" (Fir) |
| (I) 2 | 5.8 | 17.5 | 5.6 | 47.0 | "yezomatsu" (Spruce) |
| (I) 3 | 5.4 | 20.0 | 5.4 | 62.0 | "shina" (Basswood) |
| (I) 4 | 5.5 | 20.0 | 5.2 | 62.0 | "nara" (Oak) |
| (II) 1 | 6.0 | 14.5 | 5.4 | 90.0 | "todomatsu" |
| (II) 2 | 5.2 | 11.5 | 5.2 | 62.0 | "yezomatsu" |
| (II) 3 | 5.4 | 20.0 | 5.4 | 54.0 | "shina" |
| (II) 4 | 5.4 | 23.5 | 5.2 | 160.0 | "nara" |
| (III) 1 | 5.2 | 9.0 | 5.2 | 90.0 | Brown rotted "hinoki" |
| (III) 2 | 4.6 | 3.0 | 4.6 | 130.0 | White rotted "yezomatsu" |
| (III) 3 | 5.0 | 17.5 | 5.0 | 145.0 | " |
| (III) 4 | 5.2 | 20.0 | 5.2 | 62.0 | " |

* Mark in Fig. 1.

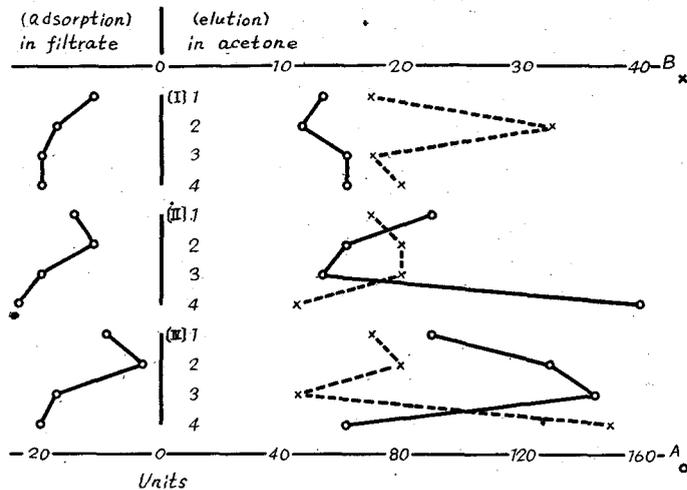


Fig. 1. Adsorption and elution of penicillin with hydrated active charcoals from most common woods in Hokkaido. (connecting lines have no special meaning but to help perception)

With 37 samples which had been prepared previously and described in the previous report³⁾ (Experiment No. (I) 1, 2, 3, 4, (II) 1, 2, 3, 4, (III) 1, 2, 3, 4, (S) 1, 2, 3, 4, (IV) 1, 2, 3, 4, (V) 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and (VII) 1, 2, 3, 4, 5.) potency of penicillin was tested by the same method as in experiment (A). But in this case the potency of penicillin in the filtrates after adsorption could not be assayed on account of mistakes in the dilution of filtrates, therefore, only potencies of acetone solutions were determined. The results are indicated in Table 3. The potency

Table 3. Elution of penicillin with H-A charcoals

| Exp. B. ×* | | | |
|--------------------------------|--|-----------|-------------------|
| Crude penicillin culture fluid | pH | Potency | Average |
| at beginning | 8.6 | 42.5 unit | |
| in the middle of exp. | 8.6 | 27.0 | 31.0 unit |
| after experiment | 8.6 | 23.5 | |
| Charcoal Exp. No. | Note | pH | Potency (unit) |
| (I) 1 | "todomatsu" (Fir) | 5.4 | 17.5 |
| (I) 2 | "yezomatsu" (Spruce) | 5.4 | 32.5 |
| (I) 3 | "shina" (Basswood) | 5.4 | 17.5 |
| (I) 4 | "nara" (Oak) | 5.2 | 20.0 |
| (II) 1 | "todomatsu" | 5.4 | 17.5 |
| (II) 2 | "yezomatsu" | 5.4 | 20.0 |
| (II) 3 | "shina" | 5.2 | 20.0 |
| (II) 4 | "nara" | 5.2 | 11.5 |
| (III) 1 | Brown rotted "hinoki" | 5.2 | 17.5 |
| (III) 2 | White rotted "yezo" | 5.0 | 20.0 |
| (III) 3 | " | 5.2 | 11.5 |
| (III) 4 | " | 5.0 | 37.5 |
| (III) 5 | Br. rotd. "karamatsu" | 5.2 | 27.0 |
| (S) 1 | Merck animal | 7.2 | 20.0 |
| (S) 2 | Ta | 7.4 | 49.0 |
| (S) 3 | A | 7.6 | 32.5 |
| (S) 4 | B | 7.0 | 20.0 |
| (IV) 1 | "nara", 60%—H ₂ SO ₄ | 5.4 | 37.5 |
| (IV) 2 | " 65% | 5.2 | 14.5 |
| (IV) 3 | " 70% | 5.4 | 27.0 |
| (IV) 4 | " 75% | 5.4 | 11.5 |
| (IV) 5 | " 80% | 5.0 | 14.5 |
| (V) 1 | "nara" { 6 hr | 5.2 | 27.0 |
| (V) 2 | " 65% { 8 " | 5.2 | 9.0 |
| (V) 3 | " { 10 " | 5.5 | 9.0 |
| (V) 4 | " { 6 hr | 5.2 | 17.5 |
| (V) 5 | " 70% { 8 " | 5.0 | 17.5 |
| (V) 6 | " { 10 " | 5.0 | 27.0 |
| (V) 7 | " { 6 hr | 5.2 | 17.5 |
| (V) 8 | " 75% { 8 " | 5.0 | 42.5 |
| (V) 9 | " { 10 " | 5.6 | 17.5 |
| (V) 10 | " | 5.2 | 11.5 |
| (VII) 1 | Mandarin orange peel | 5.2 | 20.0 |
| (VII) 2 | " | 5.2 | 20.0 |
| (VII) 3 | Butanol pulp | 5.2 | 14.5 |
| (VII) 4 | Extracted "nara" | 5.0 | 27.0 |
| (VII) 5 | Unextracted "nara" | 5.0 | 14.5 |

* Mark in Fig. 1

of the crude culture fluid in this experiment was lower than in experiment (A). The average figures of duplicate assays were given in the table.

From these results, charcoals (I)2, (II)4, (III)3 and (III)4 showed results opposite to those seen in experiment (A) in which the content of penicillin in the crude culture fluid was found to be higher than in this experiment (B). It seems that the other constituents of the crude culture fluid may exert influence upon the adsorption and elution of penicillin, but that can not be clarified without further investigation.

(4) Influence of wood species as source of H-A charcoal upon the adsorption and elution of penicillin

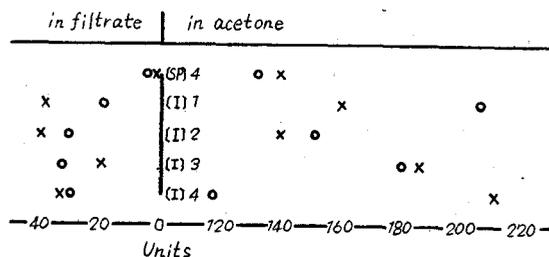
Experiments C and D. Duplicate experiments were performed to examine whether the species of wood supplying material for the charcoal had any influence upon adsorption and elution of penicillin. Use was made of samples (I)1, 2, 3, 4, and (S)4.

In this experiment 10 ml of the culture fluid were employed and treated with 0.2g dry equivalent weight of the moistened charcoal

Table 4. and Fig. 2. Adsorption and elution of penicillin with H-A charcoals

| Crude penicillin culture fluid | | pH | Potency* | Average | | | | |
|--------------------------------|----------------|-------------------|---------------|--------------------|----------------|-------------------|---------------|--------------------|
| at beginning | | 7.6 | 272.5 unit | | | | | |
| after experiment | | 7.6 | 223.8 | 248.2 unit | | | | |
| Charcoal Exp. No. | Exp. C ○ | | | | Exp. D × | | | |
| | P. in filtrate | | P. in acetone | | P. in filtrate | | P. in acetone | |
| | pH | Potency (unit) | pH | Potency* (unit) | pH | Potency (unit) | pH | Potency* (unit) |
| (Sp) 4 Carbon B | 6.2 | 4.0 | 5.8 | 132.5 | 6.3 | 0.0 | 5.8 | 140.0 |
| (I) 1 "todo" | 5.2 | 18.5 | 5.0 | 206.3 | 5.2 | 37.5 | 5.2 | 160.0 |
| (I) 2 "yezo" | 5.4 | 30.0 | 5.0 | 151.3 | 5.2 | 40.0 | 5.2 | 140.0 |
| (I) 3 "shina" | 5.2 | 32.5 | 5.2 | 180.0 | 5.0 | 20.0 | 5.0 | 186.3 |
| (I) 4 "nara" | 4.8 | 30.0 | 5.0 | 117.5 | 5.0 | 32.5 | 5.1 | 211.3 |

* the average unit of potency tested by two pieces of filter paper.



samples; and the adsorbed penicillin was eluted with 10 ml of aqueous acetone. The results of the experiments are given in Table 4 and Fig. 2. The average potency of penicillin in the culture fluid which was used in these experiments was as high as 248.2 units. In these figures (I)1 and (I)4 in elution and (I)1 and (I)3 in filtrate there were remarkable differences in the duplicate test.

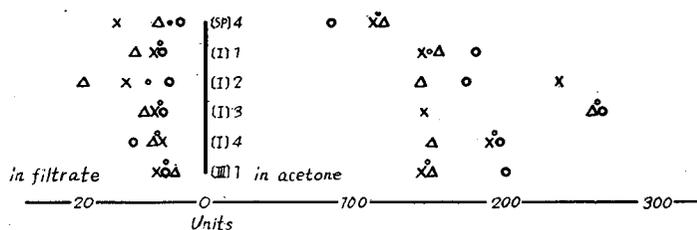
Experiments E, F, and G. In the previous duplicate test wide differences were found between the two values in the same charcoal, because the reaction had not proceeded uniformly. Then triplicate experiments were repeated with the same samples in experiments C and D, and additional (III)1 "hinoki" (brown rotted) which had been adsorbed methylene blue well but not caramel. Conditions of the experiments were the same as in experiments C and D, the results are recorded in Table 5 and Fig. 3.

From these results, it may be seen that these five charcoals adsorbed penicillin from crude culture fluid with almost the same power, (I)2 was somewhat inferior. For elution, the highest potency was given by (I)3

Table 5. and Fig. 3. Adsorption and elution of penicillin with H-A charcoals and ordinary commercial active carbon

| Crude penicillin culture fluid | | pH | | Potency | | Average | | | | | | | | |
|--------------------------------|----------|-----------------|---------|-----------------|----------|-----------------|---------|-----------------|----------|-----------------|---------|-----------------|--------------------------------------|-----------------|
| at beginning | | 7.0 | | 412.5* unit | | 380.0 unit | | | | | | | | |
| after experiment | | 7.0 | | 347.5* | | | | | | | | | | |
| Charcoal | Exp. E ○ | | | | Exp. F × | | | | Exp. G △ | | | | Average of two closely agreeing data | |
| | Filtrate | | Acetone | | Filtrate | | Acetone | | Filtrate | | Acetone | | Filt-rate | Acetone |
| Exp. No. | pH | Po-tency (unit) | pH | Po-tency (unit) | pH | Po-tency (unit) | pH | Po-tency (unit) | pH | Po-tency (unit) | pH | Po-tency (unit) | Po-tency (unit) | Po-tency (unit) |
| (Sp) 4 Active carbon B | 6.0 | 4.0 | 5.6 | 84.0 | 6.0 | 14.5 | 5.6 | 111.0 | 6.0 | 7.8 | 5.6 | 115.0 | 5.9 | 113.0 |
| (I) 1 "todo" | 4.6 | 7.3 | 4.8 | 180.0 | 4.6 | 7.8 | 4.8 | 142.5 | 4.6 | 11.5 | 4.6 | 157.5 | 7.6 | 150.0 |
| (I) 2 "yezo" | 4.7 | 5.8 | 5.0 | 172.5 | 4.8 | 13.0 | 5.2 | 235.0 | 4.7 | 20.0 | 5.2 | 142.5 | 9.4 | 157.5 |
| (I) 3 "shina" | 4.6 | 6.8 | 4.8 | 262.5 | 4.6 | 7.3 | 4.8 | 145.0 | 4.6 | 9.0 | 4.8 | 260.0 | 7.1 | 261.8 |
| (I) 4 "nara" | 4.6 | 11.5 | 4.8 | 195.0 | 4.7 | 7.8 | 5.0 | 192.5 | 4.6 | 7.8 | 5.0 | 152.5 | 7.8 | 193.8 |
| (III) 1 Brown rotted "hinoki" | 4.6 | 6.2 | 5.0 | 200.0 | 4.6 | 7.8 | 5.0 | 145.0 | 4.6 | 5.0 | 5.0 | 150.0 | 5.6 | 147.5 |

* average value of duplicate test.



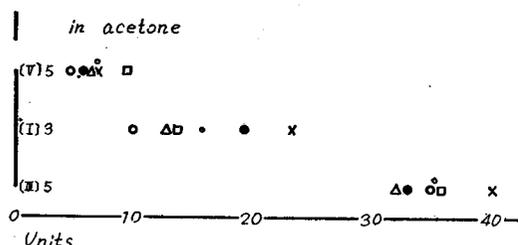
from "shina" wood, next was (I)4 from "nara" wood, then (I)2 "yezomatsu", and then (I)1 "todomatsu", (III)1 "hinoki" and (S)4 commercial active carbon. The fact that the hydrated active charcoals made from hardwood eluted penicillin well is in agreement with the results of previous tests on adsorption of methylene blue and caramel. It seems that there is no obvious relation between adsorption and elution of penicillin, however, among the commercial active carbons lately tested, the carbon that adsorbed penicillin well also eluted it well. Therefore there are some differences between the properties of the usual active carbons and the hydrated active charcoals.

(5) **Relation of adsorption and elution of penicillin to the methylene blue and caramel tests**

Experiments H, I, J, K, and L. In order to make clear the relation of adsorption and elution of penicillin to the methylene blue and caramel tests, (V)5 "nara" sample that has an excellent adsorptive power, (III)5 brown rotted "karamatsu" sample that has an inferior adsorptive power and (I)3 "shina" that has a moderate adsorptive power, were chosen and examined with penicillin. In these experiments, an aqueous solution of purified penicillin was used instead of crude culture fluid, to avoid obscurities caused by the influence of impurities. Ten ml of purified penicillin solution (average potency was 101.3 units) and 0.1 g equivalent

Table 6. and Fig. 4. Elution of penicillin with three H-A charcoals that have best, moderate and worst adsorptive power in methylene blue and caramel test

| Purified penicillin solution | | pH | Potency | Average | | | | | | | |
|---------------------------------|----------|----------------|-----------|----------------|----------|----------------|----------|----------------|----------|----------------|----------------|
| at beginning | | 5.8 | 71.8 unit | 101.3 unit | | | | | | | |
| after experiment | | 5.8 | 130.8 | | | | | | | | |
| Charcoal Exp. No. | Exp. H ○ | | Exp. I × | | Exp. J △ | | Exp. K □ | | Exp. L ● | | Average |
| | pH | Potency (unit) | pH | Potency (unit) | pH | Potency (unit) | pH | Potency (unit) | pH | Potency (unit) | Potency (unit) |
| (V)5 "nara" | 4.8 | 4.0 | 4.6 | 7.3 | 4.6 | 7.3 | 4.8 | 9.0 | 4.7 | 6.8 | 7.0 |
| (I)3 "shina" | 4.8 | 10.0 | 4.8 | 23.5 | 4.8 | 13.0 | 4.7 | 13.0 | 4.7 | 18.5 | 15.6 |
| (III)5 Brown rotted "karamatsu" | 4.7 | 35.0 | 5.0 | 40.0 | 4.8 | 32.5 | 4.9 | 35.0 | 4.8 | 32.5 | 35.0 |



amount of charcoal were used and handled by the same procedure as in the above experiments described in present report.

As there were no impurities in the testing solution, the adsorption of penicillin by the charcoal was done so perfectly that no penicillin was found in the filtrate after adsorption. Therefore the assay was made only with the eluted penicillin in the dilute acetone solution. The experiments were repeated five times, and the results are recorded in Table 6 and Fig. 4.

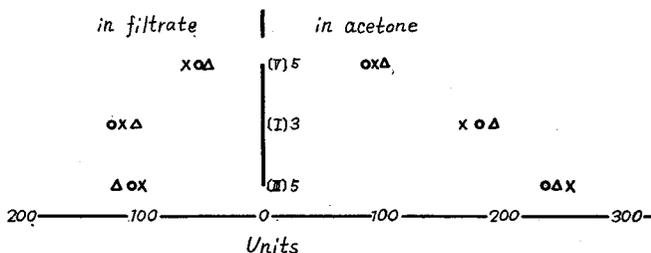
Experiments M, N, and O. It will be noted from these results that the hydrated active charcoal which adsorbed methylene blue and caramel well eluted less penicillin, it seems that the charcoal adsorbed penicillin and held it strongly, and therefore with difficulty eluted it. In order to determine the adsorption order of these charcoal, the potency of the testing penicillin solution was increased so that the penicillin might surely be found in the first filtrate after the adsorption.

The experiment was repeated three times using just the same procedure as in the preceding experiments. The results are given in Table 7 and Fig. 5.

Table 7. and Fig. 5. Adsorption and elution of purified penicillin with three H-A charcoals that have best, moderate and worst adsorptive power in methylene blue and caramel test

| Purified penicillin solution | | pH | Potency* | Average | | | | | | | | | | |
|----------------------------------|----------|-----------------|------------|-----------------|----------|-----------------|---------|-----------------|----------|-----------------|---------|-----------------|-----------------|-----------------|
| at beginning | | 5.8 | 337.5 unit | | | | | | | | | | | |
| after experiment | | 5.8 | 337.5 | 362.5 unit | | | | | | | | | | |
| Charcoal | Exp. M ○ | | | | Exp. N × | | | | Exp. O △ | | | | Average | |
| | Filtrate | | Acetone | | Filtrate | | Acetone | | Filtrate | | Acetone | | Filt-rate | Acetone |
| Exp. No. | pH | Po-tency (unit) | pH | Po-tency (unit) | pH | Po-tency (unit) | pH | Po-tency (unit) | pH | Po-tency (unit) | pH | Po-tency (unit) | Po-tency (unit) | Po-tency (unit) |
| (V) 5 "nara" | 5.5 | 51.8 | 4.8 | 90.0 | 5.5 | 63.0 | 4.8 | 95.0 | 5.4 | 48.0 | 4.8 | 95.0 | 54.3 | 93.3 |
| (I) 3 "shina" | 5.4 | 127.5 | 4.8 | 180.0 | 5.6 | 120.0 | 4.7 | 167.5 | 5.3 | 105.0 | 4.8 | 190.0 | 117.5 | 179.2 |
| (III) 5 "Brown rotted karamatsu" | 5.4 | 108.8 | 5.0 | 235.0 | 5.4 | 108.0 | 4.7 | 252.5 | 5.6 | 123.8 | 4.7 | 235.0 | 113.8 | 240.8 |

* Potency is the average value of duplicate assays.

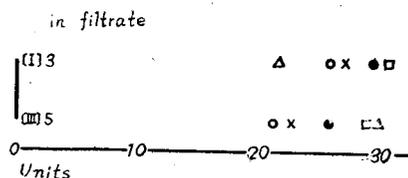


The potencies of penicillin in acetone solutions in these experiments showed the same inclination as in the preceding experiments (H, I, J, K, and L), while, as shown by filtrate, (V)5 adsorbed best, and (I)3 was a little inferior to (III)5. It appears from these results that adsorption of penicillin runs parallel with the adsorption of methylene blue and caramel, and the more penicillin is adsorbed the less it is eluted with dilute acetone.

Experiments P, Q, R, S, and T. The same experiments were repeated an additional five times with (I)3 "shina" and (III)5 brown rotted "karamatsu". The adsorptive powers of these samples are given in Table 8 and Fig. 6.

Table 8. and Fig. 6. Adsorption of penicillin with two H-A charcoals that have moderate and worst adsorptive power in methylene blue and caramel test

| Purified penicillin solution | | pH | | Potency | | Average | | | | | |
|------------------------------------|----------|-------------------|----------|-------------------|----------|-------------------|----------|-------------------|----------|-------------------|------------------------------|
| at beginning | | 6.8 | | 350.0 units | | 310.0 units | | | | | |
| after experiment | | 6.8 | | 270.0 | | | | | | | |
| Charcoal Exp. No. | Exp. P ○ | | Exp. Q × | | Exp. R △ | | Exp. S □ | | Exp. T ● | | Average Potency (unit) |
| | pH | Potency (unit) | |
| (I)3 "shina" | 5.4 | 25.7 | 5.4 | 27.0 | 5.4 | 21.6 | 5.4 | 30.5 | 5.4 | 29.3 | 26.8 |
| (III)5 Brown rotted "karamatsu" | 5.3 | 21.0 | 5.4 | 22.5 | 5.4 | 29.3 | 5.4 | 28.8 | 5.4 | 25.6 | 25.4 |



The difference in the potencies of penicillin in the filtrate between (I)3 and (III)5 were not very large in these experiments.

From these results it seems that the relations among methylene blue test, caramel test, penicillin adsorptive test and penicillin elutive test are as follows:

| | Best | Moderate | Worst |
|---------------------------------|---------|----------|---------|
| Methylene blue and caramel test | (V) 5 | (I) 3 | (III) 5 |
| Penicillin-adsorptive test | (V) 5 | (III) 5 | (I) 3 |
| Penicillin-elutive test | (III) 5 | (I) 3 | (V) 5 |

Therefore it may be said that the adsorption of penicillin runs not always parallel with the results of methylene blue and caramel test, while the more penicillin is adsorbed the less it is eluted with aqueous acetone.

However, it may be difficult to determine the suitability of the

active charcoals to the purification of penicillin by means of methylene blue or caramel test only, and without adsorption and elution test with penicillin. This conclusion may adopted not only to the hydrated active charcoal but also to the general active carbons. From only a few data it may be dangerous to make such an assertion.

(6) The influences of drying and remoistening
the charcoal upon the activity

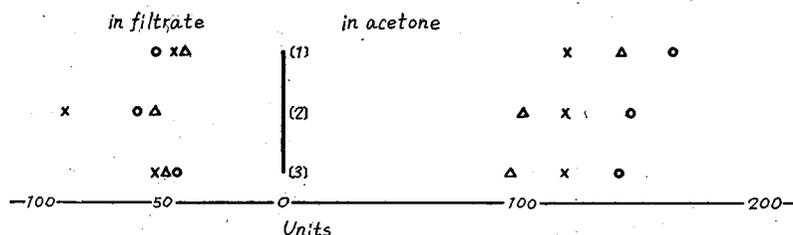
Experiments U, V, and W. Hydrated active charcoal has activity in the moistened state which is lost when the charcoal is dried⁹. Sample (I) 4 "nara" was dried in an electric oven at 105°, and tested with methylene blue solution. Methylene blue was not adsorbed by this dried charcoal. This held true even if it was remoistened (0.1 g equivalent amount was added to 10 ml or 15 ml of 0.15 per cent methylene blue solution). These samples were also tested with purified penicillin solution. The experiments were repeated three times which the results were given in Table 9 and Fig. 7.

According to these results it is remarkable that the remoistened charcoal recovers adsorptive power for penicillin even though it elutes very poorly.

Table 9. and Fig. 7. Adsorption and elution of purified penicillin with dried and remoistened H-A charcoal

| Charcoal | Purified penicillin solution at beginning | | pH | | Potency | | Average | | | | | | | |
|----------------|---|-----------------|---------|------------------|------------|-----------------|------------|------------------|----------|------------------|---------|------------------|-----------------|-----------------|
| | after experiment | | 5.7 | | 350.0 unit | | 310.0 unit | | | | | | | |
| Exp. No. | Exp. U ○ | | | | Exp. V × | | | | Exp. W △ | | | | Average | |
| | Filtrate | | Acetone | | Filtrate | | Acetone | | Filtrate | | Acetone | | Fil-trate | Acetone |
| | pH | Po-tency (unit) | pH | *Po-tency (unit) | pH | Po-tency (unit) | pH | *Po-tency (unit) | pH | *Po-tency (unit) | pH | *Po-tency (unit) | Po-tency (unit) | Po-tency (unit) |
| (1) Moistend | 5.4 | 52.5 | 5.0 | 161.3 | 5.4 | 45.3* | 4.9 | 117.5 | 5.4 | 44.0 | 5.1 | 140.0 | 47.3 | 139.6 |
| (2) Dried | 6.2 | 60.0 | 4.8 | 143.8 | 5.2 | 90.0 | 4.8 | 117.5 | 5.2 | 52.5 | 5.1 | 100.0 | 67.5 | 120.4 |
| (3) Remoistend | 5.3 | 43.5 | 4.8 | 133.8 | 5.2 | 52.5* | 4.9 | 118.8 | 5.3 | 48.8 | 5.0 | 93.8 | 48.3 | 115.5 |

* average of duplicate assays.



(7) Comparison with commercial active carbons

Experiments X, Y, and Z. In order to compare the hydrated active charcoal with the commercial general active carbons, five active carbons which had been stored in the factory of Sapporo Pharmaceutical Plant and the H-A charcoal (V) 7 "nara" were tested.

Moisture content of these commercial active carbon and H-A charcoal were as follows:

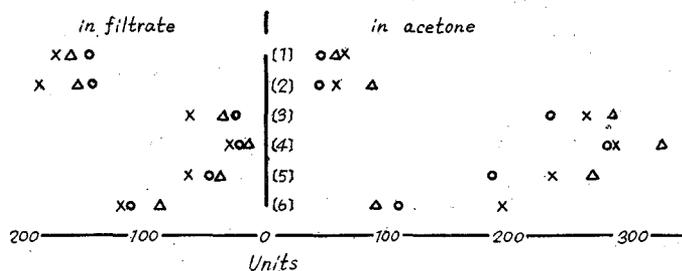
| Mark of carbon | Moisture content (%) | 0.1 g equivalent weight (g) |
|----------------|----------------------|-----------------------------|
| (1) Iz | 13.62 | 0.1158 |
| (2) Ni | 15.59 | 0.1184 |
| (3) Sh | 21.20 | 0.1269 |
| (4) Ta | 9.73 | 0.1113 |
| (5) To | 21.13 | 0.1266 |
| (6) (V) 7 | 79.80 | 0.4950 |

Each 0.1 g equivalent weight of carbon was placed in ca. 100 mℓ flask and 20 mℓ of crude penicillin culture fluid was added. The mixture was shaken by hand for a while and added with a small amount of 10 per cent sulphuric acid so that the pH value of the solution decreased to 5.0 (by Toyo pH test-paper), and was then shaken for 10 minutes on a shaking machine. Mixture was filtered, the first few mℓ of the filtrate was discarded and the remainder was taken for assay of the potency of penicillin. The charcoal that adsorbed penicillin and the filter paper were placed in a bottle with 10 mℓ of 80 per cent acetone, bottle was shaken by hand and ca. 20 per cent aqueous ammonia added to make

Table 10. and Fig. 8. Adsorption and elution of penicillin with commercial active carbons and H-A charcoal

| Crude penicillin culture fluid | | pH | *Potency | Average | | | | |
|--------------------------------|-------------------------|------------------------|-------------------------|------------------------|-------------------------|------------------------|-------------------------|------------------------|
| at beginning | | 7.8 | 191.7 unit | 189.2 unit | | | | |
| after experiment | | 7.8 | 186.7 | | | | | |
| Charcoal Exp. No. | Exp. X ○ | | Exp. Y × | | Exp. Z △ | | Average | |
| | * P. in filtrate (unit) | * P. in acetone (unit) | * P. in filtrate (unit) | * P. in acetone (unit) | * P. in filtrate (unit) | * P. in acetone (unit) | * P. in filtrate (unit) | * P. in acetone (unit) |
| (1) Iz | 147.0 | 45.0 | 172.5 | 63.0 | 160.0 | 60.0 | 159.8 | 56.0 |
| (2) Ni | 141.0 | 43.0 | 187.5 | 57.8 | 152.5 | 85.5 | 160.3 | 62.1 |
| (3) Sh | 25.2 | 235.0 | 62.0 | 267.5 | 32.0 | 285.0 | 39.7 | 262.5 |
| (4) Ta | 21.2 | 280.0 | 24.5 | 285.0 | 19.0 | 325.0 | 21.6 | 296.7 |
| (5) To | 48.0 | 185.0 | 65.0 | 235.0 | 42.0 | 267.5 | 51.7 | 229.2 |
| (6) (V) 7 | 116.3 | 117.5 | 117.5 | 192.5 | 87.5 | 89.0 | 107.1 | 129.7 |

* average potency of duplicate assays.



the pH value of the solution up to 7.2 (by Toyo pH test-paper), and again shaken on a shaking machine, then filtered and the first few ml of the filtrate were discarded, with the remainder the potency of the eluted penicillin was assayed. The experiments were repeated three times and following results were obtained (Table 10 and Fig. 8).

According to these results, the order of the carbons in adsorption was (4) Ta > (3) Sh > (5) To > (6) (V) 7 > (1) Iz \cong (2) Ni, and in elution the order was

(4) Ta > (3) Sh > (5) To > (6) (V) 7 > (2) Ni > (1) Iz.

The carbons that adsorbed penicillin well, also eluted it well.

The H-A charcoal did not show any excellent result in this experiment.

Summary

Some experiments were made on the adsorption of penicillin and the elution of it with aqueous acetone, using the hydrated active charcoal (H-A charcoal) which had been prepared in the writers' laboratory.

As to the wood species, charcoal prepared from "todomatsu" (fir), "yezomatsu" (spruce), "shina" (basswood) and "nara" (oak) were tested. The charcoal from "yezomatsu" adsorbed penicillin most poorly, and the others were little better. The charcoal made from "shina" eluted penicillin best and that from "nara" followed it. Apparently the adsorption and elution of penicillin by the charcoals that were made from hardwoods were superior to those from softwoods as was also true in methylene blue and caramel test.

In order to investigate whether there is any certain relation between efficiency in adsorption and elution of penicillin and efficiency in adsorption of methylene blue and caramel, three typical samples were taken and experiments were performed. According to the results, it may be said that the adsorption of penicillin was not always in parallel with that of methylene blue and caramel. But in the elution test, charcoals which adsorbed the more methylene blue and caramel were eluted the less penicillin with aqueous acetone.

H-A charcoal loses its activity by drying and does not recover it by remoistening by addition of water in the methylene blue test. However in the experiment on the adsorption of penicillin, the activity is recovered by remoistening, even though elution becomes worse.

In comparison with the general commercial active carbons, it appears that the adsorptive and elutive power of the H-A charcoal for penicillin gains moderate position. In this experiment the carbon that adsorbed the more penicillin was the more eluted with aqueous acetone.

Literature Cited

- 1) FLEMINGA: "Penicillin" 18 (1946).
- 2) WAKAKI, S.: Penicillin (Jour. of Penicillin, Japan) 1, 30 (1947).
- 3) HANZAWA, M., and SATONAKA, S.: The Research Bulletins of the College Experimental Forests, College of Agriculture, Hokkaido University, Vol. XVII, No. 2, 439 (1955).
- 4) MIZUNO, S., and KATO, Y.: Jour. Ind. Chem. of Japan 574, 961 (1943).

摘 要

トドマツ・エゾマツ・シナ・ナラ材の鋸屑から造つた水和活性炭を使用してペニシリンの吸着及び水性アセトン液による脱着に就て実験し、原料による性能の差違、市販活性炭との比較、メチレンブルー及びキヤラメル脱色試験との相関々係、乾燥、再湿潤による性能の変化等について実験を行つた。

その結果ペニシリンの吸着についてはナラ・シナ・トドマツ材から造つたものは殆ど同じで、エゾマツ材からのものが最も悪かつた。ペニシリンの脱着は広葉樹材からのものが針葉樹材から造つたものよりも勝つてゐるようと思われるが、これはメチレンブルー及びキヤラメル試験に於ても同様である。ペニシリンの吸着はメチレンブルー及びキヤラメル脱色力と常に平行する結果は得られなかつたが、脱着についてはメチレンブルー及びキヤラメル脱色力の強いものが脱着し難い結果を示した。

水和活性炭は乾燥することによつて、メチレンブルーの脱色力を低下し、再び湿潤状態にしても回復しないが、ペニシリン試験に於ては再び湿らせることによつて吸着力は回復し、脱着力は低下する結果となつた。

水和活性炭のペニシリン吸着及び脱着性能は市販の活性炭と比較して大体中位に位し本実験に於てはペニシリンをよく吸着するものほど、水性アセトン液によつてよく脱着される結果を示した。