

# Studies on Physiologically Active Phytohormones of Ferns in Taiwan

## I. Preliminary Tests on 100 Kinds of Ferns

By

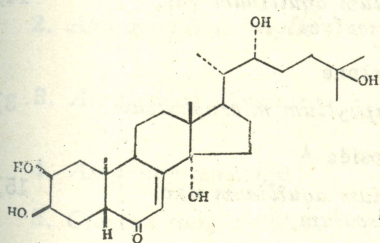
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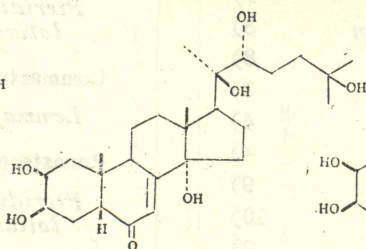
Taipei Medical College

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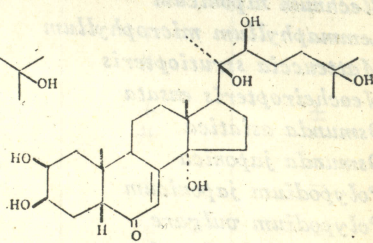
Since the isolation of ecdysterone<sup>1)</sup> from *Polypodium vulgare* in 1967 by Jizba and co-workers, many compounds exhibiting the insect moulting hormone activity have been isolated from ferns such as lemmasterone<sup>2)</sup>, ponasterone A<sup>3)7)8)</sup>, chelianthone A and B<sup>13)</sup>, ecdysone<sup>2)3)4)5)6)</sup>, shidasterone<sup>7)11)</sup>, pterosterone<sup>3)8)14)</sup>, deoxyecdysone<sup>2)</sup>, deoxycrustecdysone<sup>2)</sup>, and ponasteroside A<sup>15)</sup>. Structures of which are shown in Table 1. The rhizomes of the fern, *Polypodium* contain over 1% by dry weight of ecdysone analogues with high insect moulting hormone activity were reported<sup>14)</sup>. From this point of view, ferns are considered as the richest source of insect moulting hormones in the plant kingdom. In the past years, extensive screening survey of phytoecdysones has already been reported by Takemoto<sup>16)</sup>, Imai<sup>17)</sup> and other workers in order to look for new active compounds and rich sources. Their sources chosen for screening were representatives of a wide taxonomical distribution, they all led to a conclusion of high probability of finding active insect moulting hormones in ferns. In this paper, a chemical screening of 100 species of ferns, comprising of 17 families and 54 genera are reported. Screening results of insect moulting hormones are submitted in Table 2.



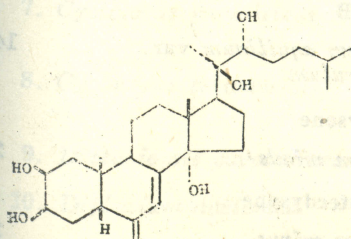
Ecdysone



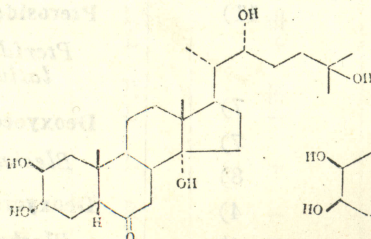
Ecdysterone (Polypodine A)



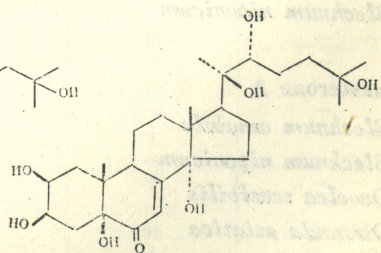
Shidasterone



Ponasterone A

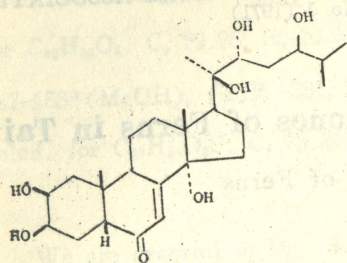


Chelianthone-A

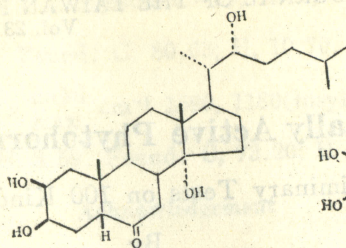


5β-hydroxyecdysterone (Polypodine-B)

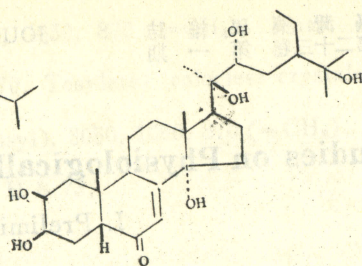




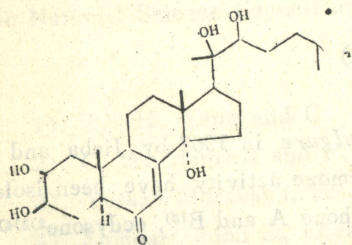
Lemmasterone



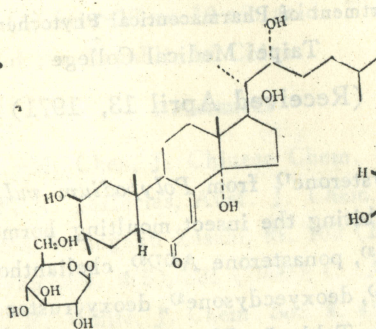
Chelianthone-B



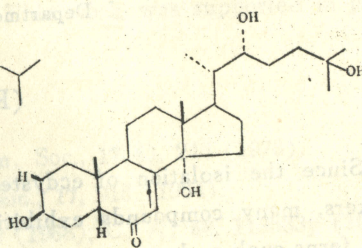
Pterosterone



Deoxycrustecdysone



Ponasteroside-A



Deoxyecdysone

|                                  | Ref. No. |                                               | Ref. No. |
|----------------------------------|----------|-----------------------------------------------|----------|
| <b>Ecdysone</b>                  |          | <b>5<math>\beta</math>-Hydroxyecdysterone</b> |          |
| <i>Blechnum minus</i>            | 2)       | <i>Polypodium vulgare</i>                     | 10)      |
| <i>Lemmaphyllum microphyllum</i> | 3)       |                                               | 12)      |
| <i>Osmunda asiatica</i>          | 4)       | <b>Chelianthone A &amp; B</b>                 |          |
| <i>Polypodium vulgare</i>        | 5)       | <i>Chelianthes tenuifolia</i>                 | 13)      |
| <i>Pteridium aquilinum</i>       | 6)       | <b>Pterosterone</b>                           |          |
| <b>Ecdysterone</b>               |          | <i>Lastrea thelypteris</i>                    | 8)       |
| <i>Blechnum amabile</i>          | 7)       | <i>Lemmaphyllum microphyllum</i>              | 3)       |
| <i>Blechnum niponicum</i>        | 7)       | <i>Onoclea sensibilis</i>                     | 8)       |
| <i>Lemmaphyllum microphyllum</i> | 3)       | <i>Pteridium aquilinum</i> var.               | 14)      |
| <i>Matteuccia struthiopteris</i> | 8)       | <i>latiusculum</i>                            |          |
| <i>Neocheiropteris ensata</i>    | 3)       | <b>Lemmasterone</b>                           |          |
| <i>Osmunda asiatica</i>          | 4)       | <i>Lemmaphyllum microphyllum</i>              | 3)       |
| <i>Osmunda japonica</i>          | 4)       | <b>Ponasteroside A</b>                        |          |
| <i>Polypodium japonicum</i>      | 9)       | <i>Pteridium aquilinum</i> var.               | 15)      |
| <i>Polypodium vulgare</i>        | 10)      | <i>latiusculum</i>                            |          |
| <i>Pleopeltis thunbergiana</i>   | 3)       | <b>Warabisterone</b>                          |          |
| <b>Shidasterone</b>              |          | <i>Pteridium aquilinum</i> var.               | 14)      |
| <i>Blechnum niponicum</i>        | 7)       | <i>latiusculum</i>                            |          |
|                                  | 11)      | <b>Pteroside B</b>                            |          |
| <b>Ponasterone A</b>             |          | <i>Pteridium aquilinum</i> var.               | 14)      |
| <i>Blechnum amabile</i>          | 7)       | <i>latiusculum</i>                            |          |
| <i>Blechnum niponicum</i>        | 7)       | <b>Deoxyecdysone</b>                          |          |
| <i>Onoclea sensibilis</i>        | 8)       | <i>Blechnum minus</i>                         | 2)       |
| <i>Osmunda asiatica</i>          | 4)       | <b>Deoxycrustecdysone</b>                     |          |
| <i>Osmunda japonica</i>          | 4)       | <i>Blechnum minus</i>                         | 2)       |



## EXPERIMENTAL

### 1. Collection and preparation of crude extracts:

The ferns were collected in Taipei Hsien, Chia-i Hsien and Ping-tung Hsien from 1968 to 1970. The leaf portion and the rhizome portion of each plant were air dried and finally fragmented and a 5-gram portion of the sample was extracted with 15 to 20 ml methanol on a water bath for 5 minutes. The filtrate was then subjected to chemical preliminary tests.

### 2. Chemical preliminary tests:

- (a) Liebermann-Burchard Reaction.: The procedures adopted in this reaction were in accordance with those described by Brieskorn and Hofman<sup>18</sup>.
- (b) Shinoda Test.: The procedures adopted in the test for flavonoids were in accordance with those described by Shinoda<sup>19</sup>. A pink to red color was considered as positive.
- (c) Mayer's Test.: The procedures adopted in the test for alkaloids were in accordance with those described by Peach and Tracy<sup>20</sup>. A creamy white precipitate was considered as positive.

## RESULT

Table 2. Results on Chemical Screening

| Name of Fern                        | Part | Chemical Tests |     |   |
|-------------------------------------|------|----------------|-----|---|
|                                     |      | S              | L-B | M |
| Angiopteridaceae                    |      |                |     |   |
| 1. <i>Angiopteris lygodiiifolia</i> | R    | —              | +   | — |
|                                     | L    | —              | +   | — |
| Aspidiaceae                         |      |                |     |   |
| 2. <i>Abacopteris triphylla</i>     | R    | —              | +   | — |
|                                     | L    | —              | +   | — |
| 3. <i>Acrophorus stipellatus</i>    | R    | ++             | +   | ± |
|                                     | L    | +              | +   | — |
| 4. <i>Athyrium arisanense</i>       | L    | —              | +   | — |
| 5. <i>Ctenitis subglandulosa</i>    | R    | +              | +   | — |
|                                     | L    | +              | +   | — |
| 6. <i>Cyclosorus atuminatus</i>     | R    | +              | +   | — |
|                                     | L    | +              | +   | — |
| 7. <i>Cyclosorus parasiticus</i>    | R    | +++            | +++ | — |
|                                     | L    | +              | +   | — |
| 8. <i>Cyclosorus taiwanensis</i>    | R    | +++            | +   | — |
|                                     | L    | —              | +   | — |
| 9. <i>Diplaziopsis cavaleriana</i>  | L    | +              | +   | — |
| 10. <i>Diplazium donianum</i>       | R    | —              | +   | — |
|                                     | L    | —              | +   | — |



|                                          |        |         |         |         |
|------------------------------------------|--------|---------|---------|---------|
| 11. <i>Diplazium esculentum</i>          | R<br>L | -<br>±  | ++<br>+ | -<br>-  |
| 12. <i>Diplazium japonicum</i>           | R<br>L | -<br>-  | +<br>+  | -<br>-  |
| 13. <i>Diplazium kawakamii</i>           | R<br>L | -<br>-  | +<br>+  | -<br>-  |
| 14. <i>Diplazium lanceum</i>             | R<br>L | +<br>-  | +<br>+  | -<br>-  |
| 15. <i>Diplazium maximum</i>             | R<br>L | ±<br>-  | +<br>+  | -<br>-  |
| 16. <i>Diplazium mettenianum</i>         | R<br>L | -<br>±  | +<br>±  | -<br>-  |
| 17. <i>Dryopteris scottii</i>            | R<br>L | -<br>±  | +<br>+  | -<br>-  |
| 18. <i>Dryopteris sieboldii</i>          | R<br>L | -<br>-  | +<br>+  | -<br>-  |
| 19. <i>Peranema cyatheoides</i>          | R<br>L | +<br>-  | +<br>+  | -<br>-  |
| 20. <i>Phegopteris decursive-pinnata</i> | R<br>L | +<br>+  | +<br>+  | -<br>-  |
| 21. <i>Rumohra amabilis</i>              | R<br>L | -<br>-  | +<br>+  | -<br>-  |
| 22. <i>Tectaria polymorpha</i>           | R<br>L | +<br>-  | +<br>+  | -<br>-  |
| 23. <i>Thelypteris gracilescens</i>      | R<br>L | ++<br>- | ++<br>+ | ++<br>- |
| 24. <i>Thelypteris japonicum</i>         | R<br>L | -<br>-  | +<br>+  | -<br>-  |
| <b>Aspleniaceae</b>                      |        |         |         |         |
| 25. <i>Asplenium ensiforme</i>           | R<br>L | -<br>++ | +<br>+  | -<br>-  |
| 26. <i>Asplenium laserpitiiifolium</i>   | R<br>L | -<br>-  | +<br>+  | -<br>-  |
| 27. <i>Asplenium nidus</i>               | R<br>L | +<br>-  | +<br>±  | -<br>-  |
| 28. <i>Asplenium trichomanes</i>         | R<br>L | +<br>+  | +<br>+  | -<br>-  |
| <b>Blechnaceae</b>                       |        |         |         |         |
| 29. <i>Blechnum niponicum</i>            | R<br>L | -<br>++ | +<br>+  | -<br>-  |
| 30. <i>Blechnum orientale</i>            | R<br>L | ++<br>+ | +<br>+  | -<br>-  |
| 31. <i>Woodwardia orientalis</i>         | R<br>L | -<br>+  | +<br>±  | -<br>-  |



Cyatheaceae

32. *Cyathea lepifera*

|   |    |    |   |
|---|----|----|---|
| R | +  | +  | - |
| L | ++ | ++ | - |

33. *Cyathea podophylla*

|   |   |   |   |
|---|---|---|---|
| L | + | + | - |
|---|---|---|---|

Davalliaceae

34. *Araiostegia parvipinnula*

|   |   |   |   |
|---|---|---|---|
| R | - | + | - |
| L | - | + | - |

35. *Araiostegia predurans*

|   |    |   |   |
|---|----|---|---|
| R | +  | + | - |
| L | ++ | + | - |

36. *Davallia divaricata*

|   |   |    |   |
|---|---|----|---|
| R | + | ++ | - |
| L | ± | ++ | - |

37. *Davallia mariesii*

|   |   |   |   |
|---|---|---|---|
| R | + | + | - |
| L | + | + | - |

38. *Nephrolepis exaltata*

|   |    |   |   |
|---|----|---|---|
| R | ++ | + | - |
| L | -  | + | - |

39. *Nephrolepis hirsutula*

|   |   |   |   |
|---|---|---|---|
| R | - | + | - |
| L | ± | + | - |

40. *Oleandra wallichii*

|   |   |    |   |
|---|---|----|---|
| R | - | ++ | - |
| L | + | +  | - |

Dipteridaceae

41. *Dipteris conjugata*

|   |    |   |   |
|---|----|---|---|
| R | ++ | + | - |
| L | +  | + | - |

Gleicheniaceae

42. *Dicranopteris dichotoma*

|   |    |   |   |
|---|----|---|---|
| R | ++ | + | - |
| L | ±  | + | - |

Hymenophyllaceae

43. *Vandenboschia auriculata*

|   |   |   |   |
|---|---|---|---|
| L | - | + | - |
|---|---|---|---|

Lycopodiaceae

44. *Lycopodium cernuum*

|   |   |    |   |
|---|---|----|---|
| R | - | ++ | - |
| L | + | ++ | - |

45. *Lycopodium clavatum*

|   |   |   |   |
|---|---|---|---|
| L | + | + | - |
|---|---|---|---|

Osmundaceae

46. *Plenasium banksiiifolium*

|   |   |    |   |
|---|---|----|---|
| R | - | ++ | - |
| L | - | ±  | - |

Plagiogyriaceae

47. *Plagiogyria formosana*

|   |    |    |   |
|---|----|----|---|
| R | ++ | +  | - |
| L | +  | ++ | - |



**Polypodiaceae**

48. *Arachniodes amabilis*
49. *Arthromeris lehmanni*
50. *Colysis elliptica*
51. *Colysis wrightii*
52. *Crypsinus engleri*
53. *Crypsinus hastatus*
54. *Crypsinus quasidivaricatus*
55. *Lemmaphyllum microphyllum*
56. *Lepisorus kawakamii*
57. *Lepisorus obligolepidus*
58. *Microsorium buergerianum*
59. *Microsorium fortunei*
60. *Microsorium membranifolium*
61. *Polypodium taiwanianum*
62. *Pseudodrynaria coronans*
63. *Pyrrosia linearifolia*
64. *Pyrrosia lingua*
65. *Pyrrosia polydactylis*
66. *Pyrrosia sheareri*

**Pteridaceae**

67. *Adiantum capillus-veneris*  
68. *Adiantum flabellatum*  
69. *Cibotium barometz*



|                                     |        |         |    |   |
|-------------------------------------|--------|---------|----|---|
| 70. <i>Coniogramme fraxinea</i>     | R<br>L | -<br>+  | +  | - |
| 71. <i>Coniogramme intermedia</i>   | R<br>L | -<br>-  | +  | - |
| 72. <i>Coniogramme japonica</i>     | R<br>L | +       | ++ | - |
| 73. <i>Ctenitis subglandiflora</i>  | R<br>L | +       | +  | - |
| 74. <i>Dennstaedtia scabra</i>      | R<br>L | +       | +  | - |
| 75. <i>Dennstaedtia scandens</i>    | L      | +       | +  | - |
| 76. <i>Histiopteris incisa</i>      | R<br>L | -<br>+  | +  | + |
| 77. <i>Microlepia hookeriana</i>    | R<br>L | -<br>-  | +  | - |
| 78. <i>Microlepia marginata</i>     | R<br>L | +       | +  | - |
| 79. <i>Odontosoria chinensis</i>    | R<br>L | -<br>+  | +  | - |
| 80. <i>Onychium japonicum</i>       | R<br>L | -<br>±  | +  | ± |
| 81. <i>Onychium siliculosum</i>     | R<br>L | -<br>-  | +  | - |
| 82. <i>Pityrogramma calomelanos</i> | R<br>L | ++<br>+ | +  | - |
| 83. <i>Pteridium aquilinum</i>      | R<br>L | +       | +  | - |
| 84. <i>Pteris ensiformis</i>        | R<br>L | +       | +  | - |
| 85. <i>Pteris longipinna</i>        | L      | -       | +  | - |
| 86. <i>Pteris semipinnata</i>       | R<br>L | +       | +  | - |
| 87. <i>Pteris tripartita</i>        | L      | ±       | +  | - |
| 88. <i>Pteris vittata</i>           | R<br>L | +       | +  | - |
| 89. <i>Pteris wallichiana</i>       | R<br>L | -       | +  | - |
| 90. <i>Sphenomeris chusana</i>      | R<br>L | -       | +  | - |
| Schizaeaceae                        |        |         |    |   |
| 91. <i>Lygodium japonicum</i>       | R<br>L | -       | +  | - |
| 92. <i>Lygodium scandens</i>        | L      | -       | +  | - |



|                                      |   |    |    |   |
|--------------------------------------|---|----|----|---|
| <b>Selaginellaceae</b>               |   |    |    |   |
| 93. <i>Selaginella delicatula</i>    | R | +  | +  | - |
|                                      | L | ++ | +  | - |
| 94. <i>Selaginella doederleinii</i>  | R | -  | +  | - |
|                                      | L | +  | +  | - |
| 95. <i>Selaginella mollendorffii</i> | R | -  | +  | - |
|                                      | L | -  | ±  | - |
| 96. <i>Selaginella remotifolia</i>   | L | ++ | +  | - |
| 97. <i>Selaginella tamariscina</i>   | R | ++ | ++ | - |
|                                      | L | +  | +  | - |
| <b>Vittariaceae</b>                  |   |    |    |   |
| 98. <i>Vittaria anguste-elongata</i> | R | -  | +  | - |
|                                      | L | -  | +  | - |
| 99. <i>Vittaria flexuosa</i>         | R | -  | +  | - |
|                                      | L | -  | +  | - |
| 100. <i>Vittaria zosterifolia</i>    | R | -  | +  | - |
|                                      | L | -  | +  | - |

Plant part: R—rhizomes

L—leaves

Chemical tests: S: Shinoda Test

L-B: Liebermann-Burchard Reaction

M: Mayer's Test

## DISCUSSION

In our chemical preliminary tests, all were positive in Liebermann-Burchard's Reaction, thus, denoting the presence of either steroid or triterpenoid substances. As we were supported by the fact that six kinds of ferns, namely, *Acrophorus stipellatus*, *Adiantum flabellatum*, *Davallia divaricata*, *Diplazium donianum*, *Diplazium esculentum* and *Plenasium banksifolia*, selected out to undergo bioassay tests from the hundred species showing evidence\* of insect moulting activities which further verify our postulation that a high probability of insect moulting hormones in relation with the positive Liebermann-Burchard's reaction can be consulted. In conjunction with previous works by Takemoto and Imai in the bioassay test, about 15% of the ferns examined possess hormonal activity in high frequency. Also, Shinoda Tests and Mayer's Test were also performed as a reference of chemotaxonomical distribution in ferns. There were 31 species showing positive signs in Flavonoid Test, however, alkaloid content was very rare, only 2 out of a hundred give positive Mayer's Test, namely, *Thelpteris gracilescens* (*Aspidaceae*) and *Histiopteris incisa* (*Pteridaceae*). Further investigations on the active constituents of insect moulting hormones are in progress. We are, therefore, in the hope that this programme would furnish late investigators a specific reference in insect-moulting hormones distribution in Taiwan.



## ACKNOWLEDGMENT

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## REFERENCES

- 1) J. Jizba, K. Slama, V. Herout, F. Sorm; *Tetrahedron Letters*, 1689 (1967)
- 2) Y. K. Chong, M. N. Galbraith, D. H. S. Horn; *J. Chem. Soc.*, (D) 1217 (1970)
- 3) T. Takemoto, Y. Hikino, T. Arai, C. Konno, S. Nebetani, H. Hikino; *Chem. Pharm. Bull.*, **16**, 759 (1968)
- 4) T. Takemoto, Y. Hikino, H. Jin, T. Arai, H. Hikino; *Chem. Pharm. Bull.*, **16**, 1636 (1968)
- 5) G. Heinrich, H. Hoffmeister; *Tetrahedron Letters*, 6083 (1968)
- 6) J. N. Kaplanis, M. J. Thompson, W. E. Robbins, B. M. Bryce; *Science*, **157**, 1436 (1967)
- 7) T. Takemoto, T. Okuyama, S. Arihara, Y. Hikino, H. Hikino; *Chem. Pharm. Bull.*, **17**, 1973 (1969)
- 8) T. Takemoto, Y. Hikino, T. Arai, M. Kawahara, C. Konno, S. Arihara, H. Hikino; *Chem. Pharm. Bull.*, **15**, 1816 (1967)
- 9) S. Imai, T. Toyosato, M. Sakai, S. Fujioka, E. Murata; *Chem. Pharm. Bull.*, **17**, 340 (1969)
- 10) G. Heinrich, H. Hoffmeister; *Experientia*, **23**, 995 (1967)
- 11) T. Takemoto, Y. Hikino, T. Ikuyama, S. Arihara, H. Hikino; *Tetrahedron Letters*, 6095 (1968)
- 12) J. Jizba, V. Herout, F. Sorm; *Tetrahedron Letters*, 5139 (1967)
- 13) A. Faux, M. N. Galbraith, D. H. S. Horn, E. J. Middleton, J. A. Thompson; *J. Chem. Soc.* (D) 243 (1970)
- 14) T. Takemoto, S. Arihara, Y. Hikino; H. Hikino; *Chem. Pharm. Bull.*, **16**, 762 (1968)
- 15) H. Hikino, S. Arihara, T. Takemoto; *Tetrahedron*, 3909 (1969)
- 16) T. Takemoto, S. Ogawa, N. Nishimoto, S. Arihara, K. Bue; *Yakugaku Zasshi*, **87**, 1414 (1967)
- 17) S. Imai, T. Toyosato, M. Sakai, S. Fujioka, E. Murata, M. Goto; *Chem. Pharm. Bull.*, **17**, 335 (1969)
- 18) C. H. Brieskorn, H. Hofman, *Arch. Pharm.*, **297**, 577 (1964)
- 19) J. Shinoda; *Yakugaku Zasshi*, **48**, 214 (1928)
- 20) K. Peach, M. V. Tracy; *Moderne Methoden der Pflanzen Analyse* Vol. IV p. 337, (1955)



# 臺灣產羊齒類生理活性植物荷爾蒙之研究

## 第一報 羊齒類一百種之預試驗

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Jizba 氏首由羊齒類單離出 Ecdysterone 及 Polypodine B 等昆蟲變態活性物質，相繼地，多種之活性物質復由不同羊齒類植物中被單離，乃引起著者研究之興趣。本報收集羊齒類 17 科，54 屬計 100 種，依植物部，葉分別作 Liebermann-Burchard's Reaction, Shinoda test 及 Mayer's test。結果 Mayer's test 呈陽性者僅 2 種，Shinoda test，根部 45 種，葉部 48 種呈陽性，而根葉部均呈陽性者計 31 種；Liebermann-Burchard's Reaction 均為陽性反應，之中根部有 9 種，葉部有 11 種之反應特別明顯，根、葉均為陽性者有 20 種。同時部分與日本東北大學藥學部 竹本常松 教授合作，經 insect moulting activities 之 bioassay test，更確實其中 *Acrophorus stipellatus* (擬鱗毛蕨)、*Adiantum flabellatum* (扇葉鐵線蕨)、*Davallia divaricata* (高山骨碎補)、*Diplazium donianum* (細柄友蓋蕨)、*Diplazium esculentum* (過溝菜蕨)、*Plenasium banksiifolium* (粗齒革葉蕨) 等六種呈陽性。