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Isolation of Cyasterone and Ecdysterone from Plant Materials[1]

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The isolations of the following substances are described: cyasterone and ecdysterone from *Ajuga decumbens* Thunb., *A. incisa* Maxim. and *A. nipponensis* Makino, and ecdysterone from *Trillium Smallii* Maxim., *T. Tschonoskii* Maxim., *Stachyurus praecox* Sieb. et Zucc. and *Polypodium japonicum* Makino.

Occurrence of insect-moulting substances in the vegetable kingdom[3-16] prompted us to investigate more than one thousand species of plants, and this had led to the discovery of more than 40 species showing the activity towards insects. The *Chilo* dipping test[17] was indispensable for the rapid assay of a great number of plant extracts, while the liquid chromatographic separation was of great help in identifying the active substances in many cases.

The present paper deals with the isolation of cyasterone (**1**) and ecdysterone (**2**) from *Ajuga decumbens* Thunb. ("kiranso" in Japanese), *A. incisa* Maxim. ("hiiragiso") and *A. nipponensis* Makino ("junihitoe"), and isolation of ecdysterone from *Trillium Smallii* Maxim. ("enreiso"), *T. Tschonoskii* Maxim. ("miyamaenreiso"), *Stachyurus praecox* Sieb. et Zucc. ("kibushi") and *Polypodium japonicum* Makino ("oshakujidenda"). Although ecdysterone occurs widely in plants, cyasterone has so far only been isolated from *Cyathula capitata* Moquin-Tandon (Amaranthaceae)[9]; it is interesting that this unique C-29 steroidal lactone has now been isolated from the Labiatae family.

Besides these, some insect-moulting substances have been assumed to be present in several plants by thin-layer chromatography. These include ecdysterone in *Athyrium yokoscence*

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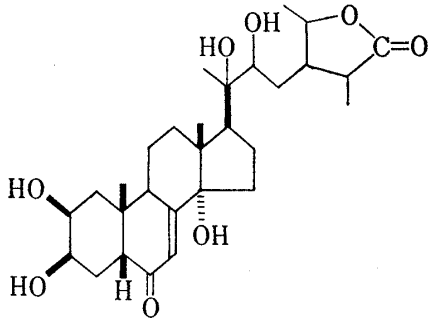
CHRIST ("hebinonegoza"), *Cyclosorus acuminatus* NAKAI ("hoshida"), *Pteris cretica* L. ("oobainomotoso"), *Osmunda japonica* THUNB. ("zenmai"), *Paris tetraphylla* A. GRAY ("tukubaneso"), *Helleborus niger* L. ("kurisumasurozu"), ponasterone A and ecdysterone in *Struthiopteris niponica* NAKAI ("shishigashira"), inokosterone and ecdysterone in *Lychnis miqueliana* ROHRB. ("fushigurosenno") and pterosterone and ecdysterone in *Lychnis chalcedonica* L. ("amerikasenno").



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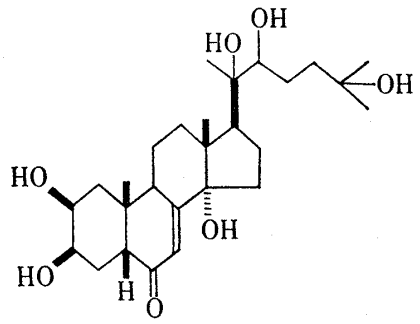

2

Experimental[18]

Material——All plants were collected in May—July, 1967.

General Extraction Procedure——The fresh plant material was soaked in five times its weight of methanol, homogenized, and the homogenate was filtered to give the methanol extract. The extraction was repeated once more, and the combined methanol extracts was concentrated and treated with water to make a 30% aqueous methanol solution. This was extracted with *n*-hexane and the hexane layer was discarded. The aqueous methanolic layer was concentrated to about half of its volume and extracted with ethyl acetate. The ethyl acetate extract was evaporated to dryness and was subjected to chromatography on silica gel. The substance (1) eluted with chloroform–methanol (9:1) was recrystallized from methanol to give colorless needles of cyasterone.[19]

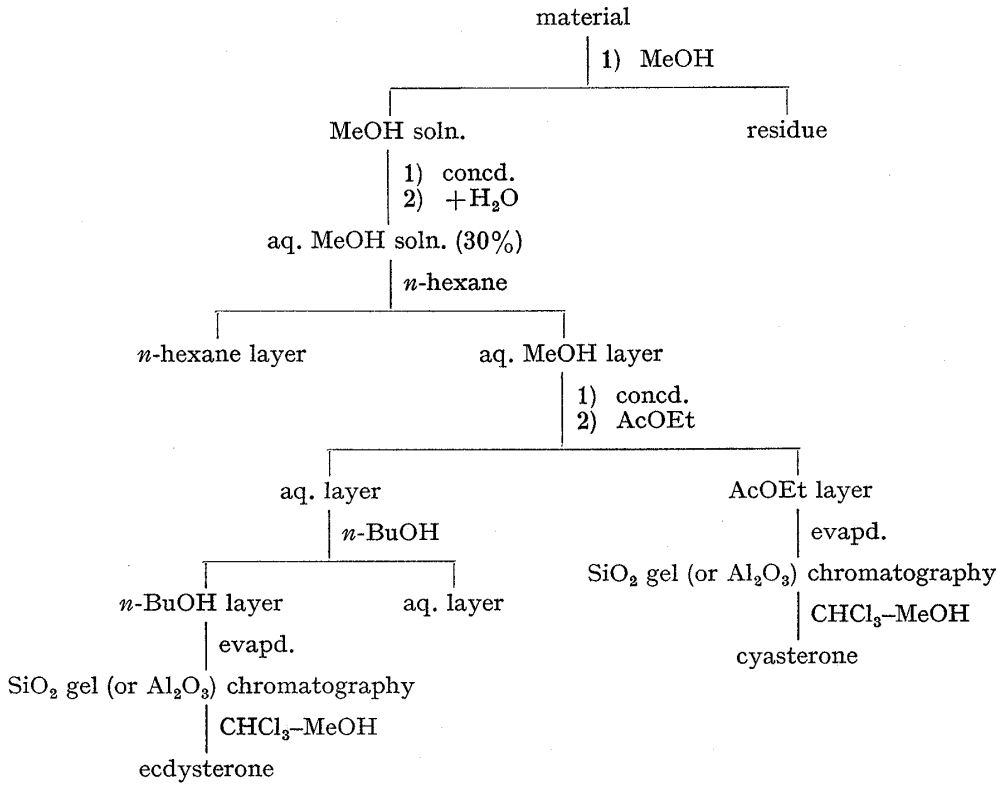

Chart 1. General Extraction Procedure

The remaining aqueous layer was reextracted with *n*-butanol and the butanol extract was chromatographed on a silica gel column. The substance (2) eluted with chloroform–methanol (4:1) was recrystallized from ethanol–ethyl acetate to give colorless needles of ecdysterone.

Identification of Cyasterone and Ecdysterone Substances (1) and (2) showed the following physicochemical properties and were identified as cyasterone and ecdysterone, respectively, which were isolated from *Cyathula capitate* Moq. and *Achyranthes japonicum* Nakai by the procedure described by Takemoto, *et al.*[4,9] The identity has also been established by preparing their acetates as summarized in the following:

Cyasterone——$C_{29}H_{44}O_8$, mp 160—162°, Mol. Wt. mass spectrum *m/e* 520 (M^+), UV λ_{max}^{EtOH} mμ (ε): 243 (12000), IR ν_{max}^{KBr} cm^{-1}: 3450 (O–H), 1750, 1660 (C=O), NMR (in deutero–pyridine) δ (ppm): 1.05 (3H, s.), 1.19 (3H, s.), 1.32 (6H, d., J=6 cps), 1.50 (3H, s.).

Cyasterone Triacetate——$C_{35}H_{50}O_{11}$, mp 238—242°, IR ν_{max}^{KBr} cm^{-1}: 3450 (O–H); 1740, 1660 (C=O), NMR (in $CDCl_3$) δ (ppm): 0.85 (3H, s.), 1.03 (3H, s.), 1.25 (3H, s.), 1.28 (3H, d., J=10 cps), 1.41 (3H, d., J=6 cps), 1.99 (3H, s.), 2.11 (6H, s.).

Ecdysterone——$C_{27}H_{44}O_7$, mp 241—242° (decomp.), UV λ_{max}^{EtOH} mμ (ε): 243 (11500), IR ν_{max}^{KBr} cm^{-1}: 3475 (O–H), 1650 (C=O), NMR (in deutero–pyridine) δ (ppm): 1.06 (3H, s.), 1.19 (3H, s.), 1.34 (6H, s.), 1.55 (3H, s.), Mol. Wt. mass spectrum *m/e* 480 (M^+).

Ecdysterone Triacetate——$C_{33}H_{50}O_{10}$, mp 147°, IR ν_{max}^{KBr} cm^{-1}: 3450 (O–H), 1745, 1660 (C=O), NMR (in $CDCl_3$) δ (ppm): 0.85 (3H, s.), 1.02 (3H, s.), 1.19 (3H, s.), 1.21 (3H, s.), 1.24 (3H, s.), 1.99 (3H, s.), 2.11 (6H, s.).

Biological Activity——Cyasterone and ecdysterone showed the following activities in the *Chilo* dipping test.[17]

Substance	Concentration	% of pupation
Cyasterone	0.003%	70 (7/10)
Ccdysterone	0.012%	70 (7/10)

Results

Our results are shown in Table I.

Table I. Contents of Phytoecdysones

Plant	Family	Part	Yield % Cyasterone	Yield % Ecdysterone
Ajuga decumbens Thunb. ("kiranso")	Labiatae	whole plants	0.008	0.012
A. incisa Maxim. ("hiiragiso")	Labiatae	whole plants	0.008	0.012
A. nipponensis Makino ("junihitoe")	Labiatae	whole plants	0.008	0.012
Trillium Smallii Maxim. ("enreiso")	Liliaceae	rhizomes	—	0.008
T. Tschonoskii Maxim. ("miyama-enreiso")	Liliaceae	rhizomes	—	0.01
Stachyurus praecox Sieb. et Zucc. ("kibushi")	Stachyuraceae	barks	—	0.06
Polypodium japonicum Makino ("oshyakujidenda")	Polypodiaceae	rhizomes	—	0.05

The Japanese names are indicated in parentheses.

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18) All melting points are uncorrected. The nuclear magnetic resonance (NMR) spectra were measured on Varian HA–100 spectrometer with tetramethylsilane as internal standard.

19) The substance (1) was once named ajugasterone by us, which later on was found to be identical with cyasterone.