pylidene-β-D-ribofuranosyl)-2-thiouracil (XI), was obtained from benzene–ethanol as a crystalline form; mp 246—247°: Mass Spectrum *m/e*: 282 (M^+): *Anal.* Calcd for $C_{12}H_{14}O_4N_2S$: C, 51.06; H, 5.00; N, 9.93; S, 11.37. Found: C, 51.05; H, 4.91; N, 10.00; S, 11.29. UV spectra (λ_{max} 243 mμ, ε 18660) are closely similar to those of 2-methylthiouridine[6] and NMR spectra are characteristic for the structure (XI) (Table I). The comparison of NMR spectra of *O*- and *S*-anhydronucleosides reveals that the signals of the proton(s) at the carbon bearing sulfur bridge are shifted to higher magnetic field by ~1 ppm.[9]

The studies of optical properties and cleavage reaction of sulfur bridge of (*S*)-anhydronucleosides are presently being undertaken.

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9) For the NMR spectra of 2,2′- and 2,5′-anhydrouridines, see M. Honjo, Y. Furukawa, M. Nishikawa, K. Kamiya, and Y. Yoshioka, *Chem. Pharm. Bull.* (Tokyo), **15**, 1076 (1967) and J. Zemlicka and F. Sorm, *Collection Czech. Chem. Commun.*, **32**, 576 (1967), respectively.

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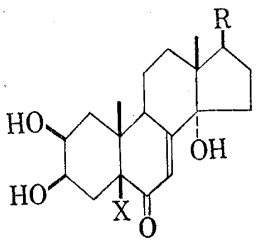
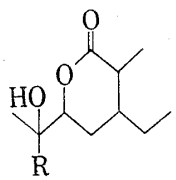
Structure of Precyasterone, A Novel C_{29} Insect-Moulting Substance from *Cyathula capitata*

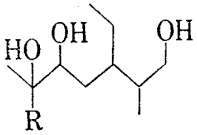
During our investigation on the roots of *Cyathula capitata* MOQUIN-TANDON (Amaranthaceae), five C_{29} phytoecdysones, sengosterone (III),[1] cyasterone (IV),[2] capitasterone (V),[3] amarasterone A (VI), and amarasterone B (VII)[4] have hitherto been isolated. In addition,



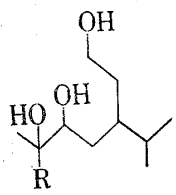
III : X=OH
IV : X=H



V : X=H



VI : X=H



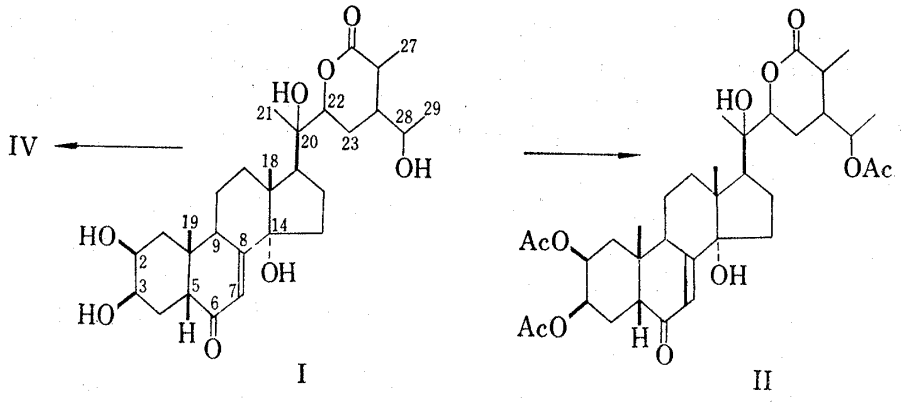
VII : X=H

there has been obtained another active C_{29} congener now named precyasterone. The present communication describes evidence which indicates the structure I for precyasterone.

1) H. Hikino, K. Nomoto, and T. Takemoto, *Tetrahedron Letters*, **1969**, 1417; *idem*, *Tetrahedron*, **26**, 887 (1970).
2) T. Takemoto, Y. Hikino, K. Nomoto, and H. Hikino, *Tetrahedron Letters*, **1967**, 3191; H. Hikino, Y. Hikino, K. Nomoto, and T. Takemoto, *Tetrahedron*, **24**, 4895 (1968).
3) T. Takemoto, K. Nomoto, Y. Hikino, and H. Hikino, *Tetrahedron Letters*, **1968**, 4929.
4) T. Takemoto, K. Nomoto, and H. Hikino, *Tetrahedron Letters*, **1968**, 4953.

Precyasterone has $C_{29}H_{44}O_8$ (M^+-H_2O at *m/e* 502), $[\alpha]_D$ +39° (MeOH). The partial-structure from C-1 to C-22 is concluded to be identical with that of capitasterone (V)[3] from 1) λ_{max} 244 nm, ν_{max} 1650 cm^{-1}, δ 6.20 ppm[5] (7-en-6-one), 2) hydrochloric acid treatment to give two products, λ_{max} 298 and 243 nm, and δ for the C-18 protons (14α-hydroxyl), 3) the ORD Cotton effect, *a* +79 (n–π^*) (A/B *cis*), 4) the shapes of the C-2 and C-3 proton signals and δ for the C-19 protons (2β,3β-dihydroxyls), and 5) the MS peaks at *m/e* 363, 345, and 327 (nucleus, 20,22-dihydroxyls).

The side-chain contains a δ-lactone ring (ν_{max} 1710 cm^{-1}) as capitasterone (V). However, the MS fragments at *m/e* 157, 139, and 121 show that the side-chain has one more oxygen than that of capitasterone (V). In fact, while capitasterone (V) gives the 2,3-diacetate, precyasterone affords a triacetate (II), whose NMR spectrum indicates the presence of the –CO–O–$C_{(22)}$H–CH_2–, a CH_3–CH<, and a CH_3–CH(OH)–CH< in the side-chain of precyasterone (Table II), the last group rationalizing the increment of one oxygen as compared with capitasterone (V) having the CH_3–CH_2–CH< instead. The chemical shifts of the C-27 protons of precyasterone and its triacetate (II) are similar to those of capitasterone (V) and its diacetate (Table I, II). Combined evidence leads to the conclusion that precya sterone has the structure I. This was confirmed by alkaline hydrolysis followed by acidification of precyasterone giving cyasterone (IV).



I II

Table I. Methyl Chemical Shifts (Pyridine)

	C-18	C-19	C-21	C-27	C-29
Capitasterone (V)[3]	1.13	1.07	1.47	1.31d	0.72t
Precyasterone (I)	1.11	1.05	1.44	1.45d	1.24d

Table II. Proton Signals ($CDCl_3$)

	C-2α	C-3α	C-7	C-9	C-18	C-19	C-21	C-22	C-27	C-28	C-29
Capitasterone 2,3-diacetate	5.06 ddd	5.34 ddd	5.89 d	3.12 ddd	0.87 s	1.03 s	1.22 s	4.20 dd	1.32 d	—	0.94 t
Precyasterone 2,3, 28-triacetate	5.07 ddd	5.33 ddd	5.88 d	3.12 ddd	0.87 s	1.03 s	1.24 s	4.18 dd	1.35 d	5.11 dq	1.27 d

5) The NMR spectra of the ecdysterols and their acetates were taken on a Varian HA-100 spectrometer in C_5D_5N and $CDCl_3$ solution, respectively. Chemical shifts are given in ppm from internal TMS. Abbreviations: s=singlet, d=doublet, t=triplet, q=quadruplet, and dd=doublet of doublets.

The hydroxy-lactone (I) which we[3] have previously postulated as a missing link in the biosynthetic pathway of the ecdysterols in this plant, has here been revealed to occur in reality.

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Biotransformation of Digitoxin by Suspension Callus Culture of *Digitalis purpurea*

Microbial transformation[1] has extensively been studied and is great significance for the synthesis of steroids and other organic compounds, but very few[2] have hitherto been reported on the biotransformation of organic compounds by plant tissue (callus) culture.

Now we wish to report the biotransformation of digitoxin to gitoxin, purpurea glycoside A and purpurea glycoside B by *Digitalis purpurea* callus tissue.

The callus tissue derived from aseptically seedling of *Digitalis purpurea* was grown on Murashige's and Skoog's agar medium containing 1 mg/liter of 2,4-D (2,4-dichlorophenoxyacetic acid) and 0.1 mg/liter of kinetin. The callus tissue was subcultured at three weeks intervals for about three years. No cardenolides are found in the callus tissue until now.

Digitoxin (total 190 mg) was administered to the suspension callus cultures of *Digitalis purpurea*. After shaking culture for 26 days, the callus (1030.3 g) were harvested and homogenized with 3 liter cold methanol in a Waring blender. The callus tissue (28.9 g dry weight) was filtered and the filtrate was evaporated under reduced pressure. The concentrated aqueous solution was extracted with *n*-hexane, chloroform and chloroform–methanol mixed solvent, respectively.

The chloroform solution was concentrated and column-chromatographyed using silica gel as adsorbent and chloroform–ethanol as developing solvent. The non-converted digitoxin was first separated and the following white substance was identified by thin-layer chromatography with authenitc gitoxin (*Rf* 0.48; chloroform: methanol=4:1 as developing solvent). Furthermore, the next following white substance (about 78 mg) was eluted out. This third substance was several times recrystallized from ethanol–ether mixed solvent. The white powder obtained (11.5 mg) showed mp 218—223° and no depression with authentic sample of purpurea glycoside A. The ultraviolet (UV) and infrared (IR) spectrum were identical with those of purpurea glycoside A. The powder was further hydrolyzed with 0.05N H_2SO_4 in 50% methanol and the hydrolysate obtained gave the same thin-layer chromatogram as that of authentic purpurea glycoside A.

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2) S.J. Stohs and E.J. Staba, *J. Pharm. Sci.*, **54**, 56 (1965); J.M.H. Graves and W.K. Smith, *Nature*, **214**, 1248 (1967).