anhydride-pyridine. Indeed the derivatized metabolite proved to be identical with I by mixed melting point, IR, NMR, and mass spectral measurements.

Treatment of the mother liquor provided the second metabolite (7 mg), mp>300°, which was similarly led to the acetate-methyl ester, mp 227°, mass spectrum (*m/e*): 586 (M^+), 317, 270. This derivative proved to be identical in all respects with the synthetic sample, methyl (17-oxoestra-1,3,5(10)-trien-3-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate,[5] mp 226.5—227.5°. Thus the structure of estrone 3-glucuronide was unequivocally assignable to this metabolite.

Further studies on the biliary estrogen metabolites which may possibly be involved in the enterohepatic circulation are being conducted and the details will be reported in the near future.

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Received March 3, 1972

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[Chem. Pharm. Bull.
20(6)1344—1347(1972)]

UDC 547.92.02 : 581.192

Components of *Metaplexis japonica* MAKINO

The structures of a number of polyoxypregnane derivatives isolated from *Metaplexis japonica* MAKINO (Japanese name: Gagaimo), a plant of Asclepiadaceae family, have been reported.[1–4] In this communication, we wish to describe the isolation and the structure of a new aglycone, ester A (1), from the same source and the structure of dibenzoyl gagaimol (III).[2]

The aglycone mixture, obtained after the mild hydrolysis of the crude glycoside,[1] was worked up by alumina column chromatography. This procedure yielded two new aglycones, ester A (I), and dibenzoylgagaimol (III). Ester A (1) shows the following data: mp 166—169° from acetone, bluish green colour with Liebermann-Burchard reaction, violet with $SbCl_3$, positive with Dragendorff reagent, negative Keller–Kiliani reaction, $[\alpha]_D^{21}=+116.2°$ ($c=1.6$, in EtOH), *Anal.* Calcd. for $C_{36}H_{43}O_8N$: C, 69.99; H, 7.02; N, 2.27. Found: C, 69.88; H, 7.09; N, 2.30), ultraviolet (UV) absorption $\lambda_{\max}^{EtOH}$ mμ (log ε): 280 (4.33), infrared (IR) absorption ν_{Max}^{Nujol} cm^{-1}: 3500, 1720, 1640, 1600, nuclear magnetic resonance (NMR) ($CDCl_3$) δ: 1.10 (19-CH_3), 1.35 (21-CH_3, d, $J=6$ Hz), 1.60 (18-CH_3), 3.50 (1H, broad m, 3α-proton), 4.85 (2H, m, 12α and 20-protons), 5.35 (1H, broad t, 6-vinylproton), 6.03 (1H, d, $J=16$ Hz, olefinic proton),

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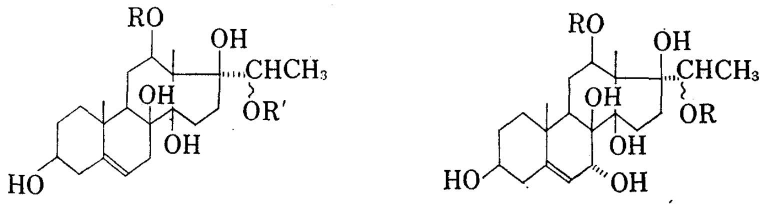
[1] H. Mitsuhashi, T. Nomura, Y. Shimizu, I. Takemori, and E. Yamada, *Chem. Pharm. Bull.* (Tokyo), **10**, 811 (1962).

[2] H. Mitsuhashi and T. Nomura, *Chem. Pharm. Bull.* (Tokyo), **13**, 274 (1965). In this report, dibenzoylgagaimol was referred to as compound (IV).

[3] H. Mitsuhashi and T. Nomura, *Chem. Pharm. Bull.* (Tokyo), **13**, 1332 (1965).

[4] H. Mitsuhashi, T. Nomura, and M. Hirano, *Chem. Pharm. Bull.* (Tokyo), **14**, 717 (1966).

7.10—7.60 (7H, m, aromatic and olefinic protons), 8.10 (1H, broad d, $J=7$ Hz, aromatic proton), 8.70 (1H, broad d, $J=5$ Hz, aromatic proton), 9.20 (1H, broad s, aromatic proton), mass spectrum *m/e*: 617 (M^+), 599 (M^+-H_2O), 494 (M^+—nicotinic acid), 469 (M^+—cinnamic acid), 346 (494—cinnamic acid), 208 (346—a, a showed a cleavage in Chart 2) 161, (b—H_2O—H, b-cleavage in Chart 2), 120 (a—H_2O), 105 (120—CH_3).⁵⁾ On the basis of these data, it is suggested that ester A is a cinnamoyl and nicotinoyl ester of polyoxygenated pregnane derivative.



I: R,R' = {nicotinoyl, cinnamoyl}
II: R,R' = H

III: R = benzoyl
IV: R = H

Chart 1

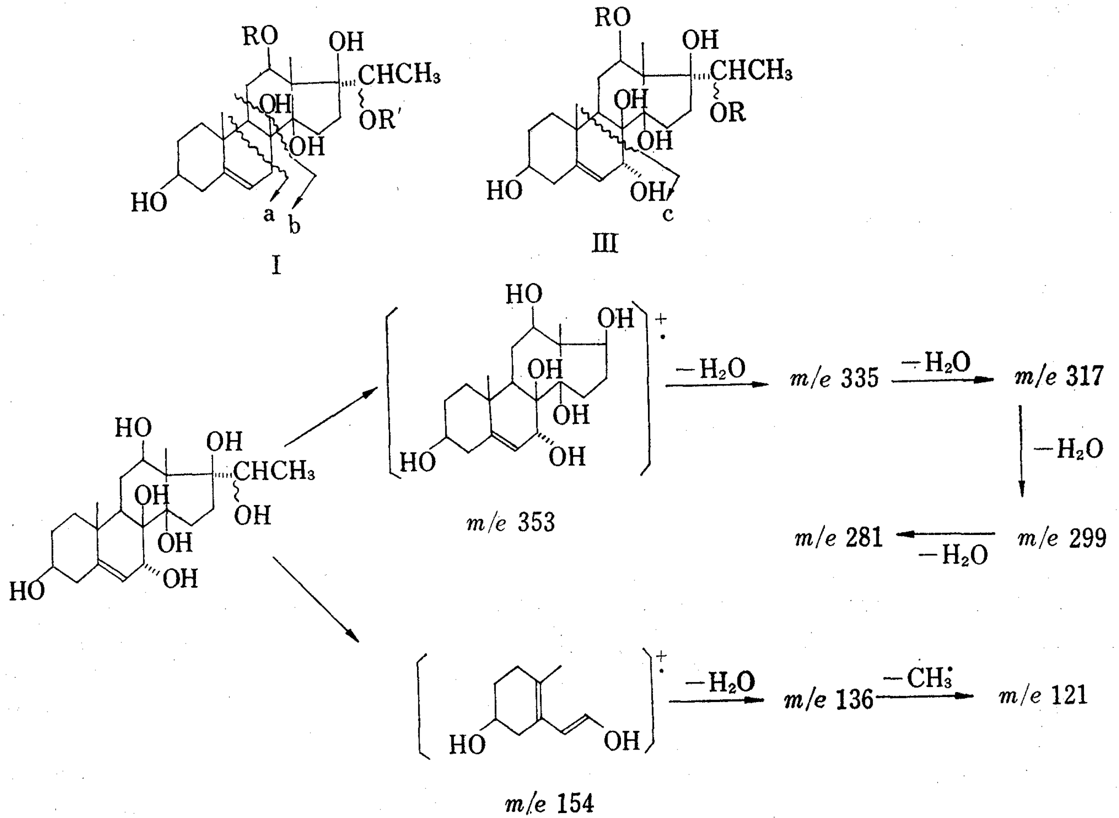


Chart 2

Hydrolysis of I with 5% methanolic potassium hydroxide gave acidic substances, which showed two spots on paper chromatography (1.5N NH_3/BuOH). From this acidic fraction, two kinds of crystalline substances were separated by means of recrystallization, which were identified with nicotinic acid and cinnamic acid respectively. A neutral product, $C_{21}H_{34}O_6$ (M^+ at *m/e* 382), was confirmed as sarcostin (II).⁶⁾ From the chemical shifts and the coupling patterns of the NMR spectrum, it is supposed that the positions of the ester linkage of

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5) M. Fukuoka, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **19**, 1469 (1971).

6) H. Mitsuhashi, Y. Shimizu, E. Yamada, T. Takemori, and T. Nomura, *Chem. Pharm. Bull.* (Tokyo), **10**, 808 (1962).

I are present at C–12 and C–20. Thus ester A is represented by the structure (I).

Dibenzoylgagaimol (III) showed mp 192—197° (from MeOH+H_2O), $[\alpha]_{20}^{589}=+26°$ (c=0.15, in MeOH, from ORD measurement), which gave colour changes reddish violet→green with a Liebermann-Burchard reaction, green with $SbCl_3$, and a negative Keller–Kiliani reaction. The molecular formula of $C_{35}H_{42}O_9$ (*Anal.* Calcd. for $C_{35}H_{42}O_9$: C, 69.29; H, 6.98. Found: C, 68.89; H, 7.27) was given for dibenzoylgagaimol. III exhibits the following spectroscopic properties, UV absorption λ_{max}^{EtOH} mμ (log ε): 229 (4.26), 273 (3.5), IR absorption ν_{max}^{Nujol} cm^{-1}: 3300. 3200, 1725, 1685, 1600, 1590, 1280, mass spectrum *m/e*: 606 (M^+), 484 (M^+—benzoic acid), 362 (M^+—2 benzoic acid), 154 (c—cleavage in Chart 2), 136 (154—H_2O), 121 (136—CH_3). These facts highly suggest that III is a dibenzoic acid ester of polyoxygenated pregnance derivative. III consumed about two moles of lead tetraacetate (in dioxane), one mole rapidly (5 hr, 0.77 mole; 23 hr, 1.30 moles), one mole slowly (95 hr, 1.84 moles). These experiments suggest the presence of α-glycol group in III.

III was hydrolyzed with 5% methanolic potassium hydroxide and this reaction mixture was separated into acid and neutral portions. Acid portion gave benzoic acid. Trituration of the neutral portion with acetone gave a new pregnane type compounds, mp 274—277°, $C_{21}H_{34}O_7$, (M^+ at *m/e* 398), for which the name gagaimol (IV) was proposed. The IR absorption at 3400 cm^{-1} (broad) indicated the presence of hydroxyl group, but there was no band assignable to a carbonyl group. The mass spectrum exhibits the ions *m/e* 154 (base peak), 136 (154—H_2O), 121 (136—CH_3), which appear to be derived from a retro-Diels-Alder reaction of the 5—6 double bond in the B ring as illustrated in Chart 2. Hence, two hydroxyl groups exist in the ring A and B, and one of them may be at C–3β from the biogenetic analogy. In comparison with the mass spectrum of III, the ester linkages are not located at the two hydroxyl groups in ring A and B. The fragment peaks (*m/e* 353, 335, 317, 299, 281) were accounted for the fragment ions (Chart 2) [M^+—45 (C_2H_5O), M^+—45—H_2O, M^+—45—2H_2O, M^+—45—3H_2O, M^+—45—4H_2O] which were also found in polyoxygenated pregnane derivatives with a glycol at C-17 and C–20.5)

The NMR spectrum of III had signals due to one vinyl-proton adjacent to a CH group (δ 5.90, 1H, d, J=5 Hz, 6-vinyl proton), two protons adjacent to a benzoxyl group (δ 5.62, 1H, q, J=5 Hz, and 10 Hz, 12α-H; δ 5.37, 1H, q, J=6 Hz, 20ξ-H), two protons adjacent to a hydroxyl group (δ 3.70, 1H, m, 3α-H; δ 4.55, 1H, d, J=5 Hz, 7β-H), a secondary methyl (δ 1.52, d, J=6 Hz, 21-CH_3), two tertiary methyl groups (δ 1.26, s, 19-CH_3; δ 2.14, s, 18-CH_3) and ten aromatic protons (δ 7.10—8.10). From this spectrum, it is supposed that III has four secondary hydroxyl groups and two of them are esterified with benzoic acid. Highly deshielded signals of δ 1.26 and 2.14 suggest the presence of C–8β hydroxyl group, with 1—3 diaxial relation to both C-18 and C–19 methyl groups. Irradiation on the proton doublet at δ 5.90 (6-vinyl proton) changes the proton doublet at δ 4.55 into a singlet, and that on the protons doublet at δ 1.52 changes the proton quartet at δ 5.37 into singlet. These decoupling experiments support the existence of the hydroxyl groups at C-7, C-8, C-17, and C-20, respectively. The coupling constants (J=5 Hz) of the proton at C-7 are in good agreement with that obtained by E. W. Garbish equation,7) and the configuration of C-7 hydroxyl group is α. The chemical shifts and the coupling constants of the NMR spectrum indicate that one benzoxyl group is present at C-12,8) the orientation of which is β and the other is present at C-20. Although there is no unequivocal evidence for the presence of 14β-hydroxyl group and the configuration at C-17, the biogenetic analogy to other natural polyhydroxy pregnanes would suggest the presence of 14β and 17β-hydroxyl group.

From the considerations above discussed, we propose for a tentative structure of dibenzoylgagaimol to the formula (III).

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7) E.W. Garbish, *J. Am. Chem. Soc.*, **86**, 5561 (1964).
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Acknowledgement We are grateful to Dr. Y. Koyama, Faculty of Pharmaceutical Sciences, Chiba University, for Mass measurement and Mrs. M. Tsuda for NMR measurement. We also thank to Mr. A. Horikawa, Mr. T. Hayashi and Miss M. Horikawa for their technical assistance.

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Received March 2, 1972

[Chem. Pharm. Bull.
20(6)1347—1348(1972)]

UDC 547.458.02.05

Isolations and Characterizations of Polysaccharides from *Tremella fuciformis* BERK[1]

The polysaccharides of basidiomycetes are studied widely,[2] but no report on *Tremella fuciformis* BERK (Shiro-kikurage) has been published until present time. We have now isolated the polysaccharides (A, B, and C) from the fruit bodies of this fungus. Their properties are described in this paper, with the aim of examining the nutritive value and the antitumor activity of these carbohydrates.

The polysaccharides were fractionated by the scheme in Chart 1. The aqueous solutions of fractions were lyophilized to yield A- and B-polysaccharides as colorless flakes and C-

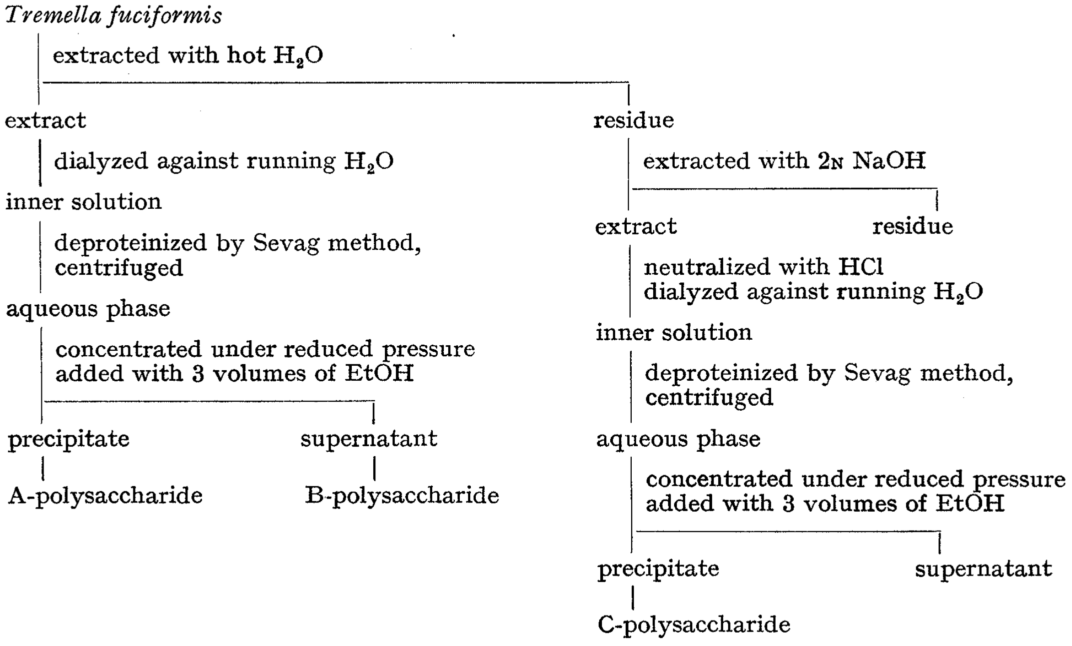

Chart 1

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