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STEROL AND LIPID COMPONENTS OF GREEN *THEA SINENSIS*

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Key Word Index—*Thea sinensis*, Theaceae tea C_{30} and C_{32} fatty alcohols, stigmasta-7 22-diene 3β-ol, stigmast-7-ene 3-β-ol, α-spinasterol gentiobioside

Abstract—Petrol extracts of green tea yielded two straight chain alcohols identified as C_{30} and C_{32} alcohols by mass spectrometry and a mixture of sterols identified as α-spinasterol and stigmast-7-ene-3-β-ol A new saponin has also been isolated from the methanol extract and shown to be α-spinasterol gentiobioside

THE PHENOLIC constituents of tea have attracted considerable attention and the colour and quality of tea as a beverage are attributed to their presence [1] Other components also may play an important part, for example, extractability and stability of tea as a beverage could depend on the presence of saponins Hashizume[2] was the first to report the occurrence of saponins in tea detected by paper chromatography and paper electrophoresis Later the crude saponin having a bitter taste was isolated from green tea (yield 15 mg/kg)[3] The presence of thea alcohols A and B,[4] carotenoids,[5] β-amyrin and α-spinasterol[6] had been reported by earlier workers Ikeda[4] suggested the molecular formula of thea alcohol *A*, m p 82–83° as $C_{32}H_{66}O$ or $C_{34}H_{70}O$, of thea alcohol *B*, m p 84–85° as $C_{28}H_{58}O$ or $C_{30}H_{62}O$ and of thea alcohol *C*, m p 92° as $C_{30}H_{62}O$ or $C_{32}H_{66}O$

In the present work, dry processed commercial green tea was extracted with light petrol, benzene, acetone and methanol in succession The petrol extract was subjected to saponification and subsequent chromatography of the neutral portion over neutral alumina The acidic portion was too small to be studied further The neutral portion yielded three compounds, *A*, *B* and *C*, the first two closely resembled thea alcohols *A* and *B*, whereas the third compound was steriodal A fraction corresponding to thea alcohol *C* was too small to be studied

Compounds *A*, m p 84–85° and *B*, m p, 86–87° were shown from IR data to be long chain fatty alcohols They formed low melting acetates which could not be purified, but they agreed with the description of thea alcohols *A* and *B*, their exact molecular formulae and structures have now been determined from their MS

[1] NATARAJAN S and SESHADRI T R (1972) *Current Science* **41**, 585 and the references cited therein
[2] HASHIZUME, A and KIYO, M D G (1962) *Shizen Kagaku* (**14**) 29, (1964) *Chem Abstr* 60 16212c
[3] HASHIZUME, A (1963) *Shizen Kagaku* (**15–16**), 53, (1964) *Chem Abstr* 60 16212b
[4] IKEDA, H (1943) *J Agr Chem Soc Japan* **19**, 301, (1952) *Chem Abstr* **46**, 1021
[5] TSUJIMURA, M (1932) *Sci Papers Inst Phys Chem Research (Tokyo)* **18**, 13–21, (1932) *Chem Abstr* **26**, 2569
[6] MATSUMOTO, T, WAINIA T and MIYAKI Y (1955) *Nippon Kagaku Zasshi* **76**, 1057, (1955) *Chem Abstr* **51**, 17969

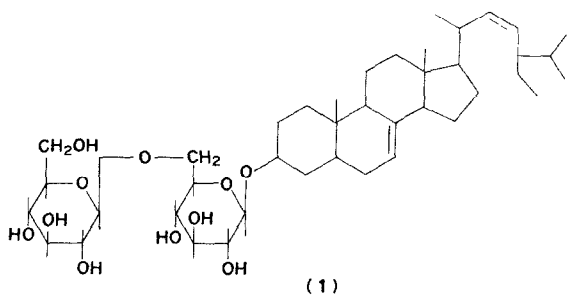
In the mass spectrum of *A* the maximum peak was at *m/e* 420 (M-H_2O) and M-(H_2O + CH_2=CH_2) peak was observed at *m/e* 392 after a further loss of C_2H_4. This was followed by the characteristic group of fragments appearing at regular intervals of 14 m.u. Further there was a peak at *m/e* 31 due to the loss of terminal-CH_2OH group. The above data clearly indicate that the compound *A* is a straight chain alcohol $Me(CH_2)_{28}CH_2OH$.

MS of *B* showed peaks at *m/e* 448 (M-H_2O) *m/e* 420 (M-46) and the expected fragments as above. The above data clearly indicate that the compound *B* is a second straight chain alcohol $Me(CH_2)_{30}CH_2OH$.

The steroidal compound *C* had m.p. 166–167° $[\alpha]_D$ + 2.2 ($CHCl_3$) and formed an acetate m.p. 176 $[\alpha]_D$ −30.1 ($CHCl_3$). The colour reactions, IR and NMR data indicated the probable identity of the compound with γ-spinasterol isolated earlier from tea leaves by Matsumoto *et al*[6] and Sakato[8]. However the MS of the acetate of compound *C* showed that it was actually a mixture of stigmast-7-en-3-β-ol and γ-spinasterol which could not be resolved by chromatography. A similar situation has recently been encountered by Clark-Lewis and Dainis[9] in the case of phytosterols isolated from several *Acacia* species.

The methanol extract of green tea was concentrated and separated into ether-soluble and -insoluble portions. The ether soluble portion on repeated purification by column chromatography yielded a homogeneous saponin, m.p. 298–300 (dec). It gave positive Liebermann-Burchard, Molisch's and froth tests and underwent acid hydrolysis to yield α-spinasterol (m.m.p. and TLC) as the genin and D-glucose as the sugar identified by comparison with authentic samples. The saponin acetate, prepared by pyridine–acetic anhydride crystallized as colourless needles from methanol m.p. 192 $[\alpha]_D$-30.1 ($CHCl_3$). The NMR of the acetate in $CDCl_3$ showed signals at δ 0.45 (3H, s, H_3C-18), 1.99, 2.18 (21H, 4s, 7-OCOMe) and 5.15 (3H, *m*, -CH=CH-, -C=CH-). One of the tertiary methyls, viz. C-18 methyl suffers an upfield shift due to shielding by the double bond at 7,8 position and it appears at δ 0.45. The aglycone sugar ratio could be determined as 1:2 from the NMR spectrum of the saponin acetate.

The exact inter-sugar linkages were established by Hakomori's permethylation method[10]. Permethylation followed by acid hydrolysis yielded two partially methylated sugars having R_G values 1 and 0.85 (solvent system for PC: *n*-BuOH: EtOH: H_2O, 5:1:4 upper layer) taking the R_G value of 2,3,4,6-tetra-*O*-methyl D-glucose as 1[11]. The faster moving spot was



(1)

[7] BUDZIKIEWICZ, H., DJERASSI, C. and WILLIAMS, D. H. (1964) *Interpretation of Mass Spectra of Organic Compounds* p. 33. Holden-Day, San Francisco.
[8] SAKATO, Y. (1942) *J. Agric. Soc. Japan* **18**, 524.
[9] CLARK-LEWIS, J. W. and DAINIS, I. (1967) *Australian J. Chem.* **20**, 1961.
[10] HAKOMORI, S. (1964) *J. Biochem.* **55**, 205.
[11] LEDERER, E. and LEDERER, M. (1957) *Chromatography* p. 249. Elsevier, New York.

identified as 2,3,4,6-tetra-*O*-methyl D-glucose and the slower one, as 2,3,4,-tri-*O*-methyl D-glucose by direct comparison with the respective authentic samples The above results indicated that the terminal glucose is attached to the 6 position of the first glucose moiety which in turn is linked to α-spinasterol This is also borne out by the observation that the glycoside did not give positive aniline hydrogen phthalate and triphenyl tetrazolium chloride tests The configuration of the glycosidic linkages was determined as β on the basis of Klyne's rule of molecular rotations [12] Hence green tea saponin is assigned the structure, β-D-glucopyranosyl (1 → 6) β-D-glucopyranosyl-3-*O*-α-spinasterol (**1**) A survey of literature shows this to be a new compound isolated for the first time from a natural source

EXPERIMENTAL

M ps were determined on a Koffler block For TLC silica gel G was used The NMR spectra were recorded on a Varian A-60 instrument using $CDCl_3$ as the solvent and TMS as internal standard The MS were recorded by direct inlet method at 70 eV ionization potential Paper chromatography was carried out on Whatman No 1 filter paper and the following solvent systems were used (a) *n*-BuOH–pyridine–H_2O (6 4 3), and (b) *n*-BuOH–EtOH–H_2O (5 1 4), upper layer

Isolation of compounds A, B *and* C Commercial green tea (4 kg) were extracted with light petrol, C_6H_6, acetone and MeOH in succession The concentrated petrol extract in C_6H_6 (100 ml) was heated with 20% methanolic KOH (100 ml) at 80° for 4 hr It was extracted with Et_2O and the extract was washed neutral with H_2O, dried (Na_2SO_4) and concentrated and the concentrate chromatographed over alumina Elution with petrol–C_6H_6 (1 3) gave compound *A*, with petrol–C_6H_6 (1 5) compound *B* and with C_6H_6–$CHCl_3$ (1 1) compound *C*

Compound A m p 84–85° It did not answer LB and TNM tests IR (KBr) 2941 2353 1538 1515 1449, 1064 727 and 717 cm^{-1} MS *m/e* 420 (M-H_2O) 392 [M-(H_2O + CH_2=CH_2)] 378 364 350 336 322 308 294 280, 266 252 238 224 210 196 182 168 154 140 126 112 98 84 70 56 42 31 and 28

Compound B m p 86–87° It did not answer LB and TNM tests IR (KBr) 2938 2350 1540 1500, 1458 1050, 727 and 720 cm^{-1} MS *m/e* 448 (M-H_2O) 420 [M-46 (H_2O + CH_2=CH_2)] 406 392 378, 364 350 336 332, 308, 294, 280, 266, 252, 238, 224, 210, 196, 182, 168, 154, 140, 126, 112 98, 84, 70, 56, 42, 31 and 28

Compound C m p 166–167°, $[\alpha]_D$ + 2 2° (*c* 0 90, $CHCl_3$) It answered LB and TNM tests IR (KBr) 2941, 1653, 1449, 1370, 1087, 1036, 966, 844, 830 and 794 cm^{-1}

Acetate of compound C Compound *C* (50 mg) was acetylated with Ac_2O–pyridine in the cold Crystallization of the product from MeOH afforded colourless needles (40 mg), m p 176°, $[\alpha]_D$ −30 1° (*c* 0 53 in $CHCl_3$) IR (KBr) 2364 1695 1653 1558 1538 1515 1504 1453 1370 1099 1036 969 848 830 and 794 cm^{-1} NMR ($CDCl_3$) δ ppm 0 45 (3H *s* H_3C-18) 0 81 (15H five Me) 2 1 (3H *s* –OCOMe) and 5 15 (3H *m*, –CH=CH–, >C=CH–) MS showed two molecular ion peaks M^+ 456 and 454 Two sets of peaks were discerned (a) *m/e* 441 (M^+–Me), 315 (M^+-side chain) 273 [M^+-(side chain +42)], 255 [M^+-(side chain + HOAc)] (b) *m/e* (M^+-Me), 411 (M^+-43), 313 (M^+-side chain − 2) and lower peaks common with (a)

Green tea saponin MeOH extract of green tea was concentrated and separated into Et_2O-soluble and Et_2O-insoluble portions The first was chromatographed over silica gel Elution with $CHCl_3$–MeOH (92 8) gave green tea saponin Fractions eluted with other higher proportions of MeOH–$CHCl_3$ gave mixtures

Green tea saponin m p 298–300°, $[\alpha]_D$ −18 5°, (*c* 0 65, pyridine) IR (KBr) 2950, 1625, 1450, 1360, 1255, 1160, 1070, 1030, 965 880, 840, 825 and 795 cm^{-1}

Hydrolysis of green tea saponin The compound (10 mg) was heated with Kiliani's mixture (1 ml) at 80° for 3 hr in a sealed tube It was extracted with Et_2O and the extract washed neutral, dried (Na_2SO_4) and evaporated The genin crystallized as needles from MeOH m p 166° It was indistinguishable from an authentic sample of α-spinasterol (m m p and coTLC, solvent system $CHCl_3$–MeOH 98 2) The aq portion was spotted along with a sample of D-glucose on Whatman No 1 paper and developed by solvent system (a) On spraying with aniline hydrogen phthalate and heating both the samples showed identical spots

Acetate of green tea saponin Green tea saponin (50 mg) was acetylated with Ac_2O–pyridine in the cold Crystallization from MeOH gave green tea saponin acetate (45 mg) m p 192° $[\alpha]_D$ −9 6° (*c* 0 707, pyridine) NMR ($CDCl_3$) δ, ppm 0 45 (3H, s, Me-18), 0 75–1 00 (15H, five Me), 1 99–2 18 (21H, 4s, 7-OCOMe) and 5 15 (3H, *m*, –CH=CH, >C=CH–) The aglycone sugar ratio was calculated to be 1 2 from the NMR spectrum

Permethylation of green tea saponin and hydrolysis NaH dispersion in oil (50%, 10 mg) was added to a solution of saponin (10 mg) in Me_2SO (2 ml) and the mixture was kept at 80° for 1 hr taking care to exclude moisture After cooling MeI (1 ml) was added and the mixture was left overnight The product was poured

[12] KLYNE, W (1950) *Biochem J* **47**, XLI

into ice H_2O and extracted with $CHCl_3$. The syrup obtained on evaporation of the solvent was dried in vacuum and permethylated once more and the resulting product which was homogeneous on TLC was hydrolysed with Kiliani's mixture (3 ml). The genin was found to be α-spinasterol (m.m.p., TLC). The mother liquor was examined for methylated sugars when 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-D-glucose were identified by direct comparison with authentic samples by paper chromatography in solvent system (b).

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