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# STRUCTURE OF CAMELLIAGENINS

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The saponin from Camellia japonica L. has been investigated by several Japanese groups including Ishidate and Takamura who isolated a sapogenol, $C_{30}H_{50}O_4$, together with tiglic acid, a uronic acid, arabinose and galactose (1); only preliminary investigations however have been reported on the structure of the sapogenol (2). We have isolated three sapogenins (3) from seeds of the same plant and established their structures, the evidence for which is described in this communication.

Crude sapogenin obtained by Ishidate's method (1) was chromatographed on silica gel to give three sapogenins: Camelliagenin A (Ia) (4), m.p. 282-283°, $[\alpha]_D$ +32.6°, camelliagenin B (Ib), m.p. 200-205°, $[\alpha]_D$ +48.0°, and camelliagenin C (Ic), m.p. 262-263°, $[\alpha]_D$ +37.1° (5). Ib, having an aldehyde group ($\nu$ 1729 cm$^{-1}$, $\delta$ 9.39 ppm), was converted to Ia by Huang-Minlon reduction and to Ic by $LiAlH_4$ reduction, thus structural correlations of the three sapogenins were established.

On acetylation with acetic anhydride and sulfuric acid, Ia and Ic gave the amorphous tetraacetate II and the amorphous pentaacetate III, respectively, both of which show no hydroxyl absorption in their IR spectra. NMR spectra (6) (Tables I and II) of Ib, II and III disclosed, besides a vinylic proton, the presence of two primary and three secondary acetoxyl groups in III, thus accounting for all the oxygen atoms in Ic. On reaction with acetic anhydride in pyridine, however, Ic produced the amorphous tetraacetate IV, $\nu$ 3520, 1740 cm$^{-1}$.

Reaction of Ic with phosgene yielded the cyclic dicarbonate V, m.p. >300°, $\nu$ 1760 cm$^{-1}$ The frequency of the carbonyl band indicated that both pairs of hydroxyl groups in Ic are in a 1,3-glycol relationship, having formed cyclic carbonates with 6-membered rings in V (7). Ic also formed a diacetonide VI, m.p. 224.5-225.5°, while Ia yielded a monoacetonide VII, m.p. 273.5-275°. The difference in chemical shift of the carbinyl

TABLE I. Lower Field Signals in NMR Spectra of Camelliagenin Derivatives

| Compds | 3α-H | 16β-H | 22β-H | 23-H | 28-H | 12-H |
|---|---|---|---|---|---|---|
| Ib | 3.3-4.2* | 4.62 br.s | 3.3-4.2* | 9.39(1H, s) | 3.3-4.2* | 5.28 m |
| II | 4.51<br>(q, 6.5, 8.5) | 5.1-5.5* | 5.1-5.5* | (Me) | 3.76(d, 11.5)<br>3.86(d, 11.5) | 5.1-5.5* |
| III | 4.81<br>(q, 7, 9) | 5.1-5.5* | 5.1-5.5* | 3.77(2H, br.s) | 3.77(2H, br.s) | 5.1-5.5* |
| IV | 4.80<br>(q, 7, 9) | 4.27 br.s | 5.33<br>(q, 7, 11) | 3.68(d, 11)<br>3.79(d, 11) | 3.70(d, 12)<br>3.91(d, 12) | 5.37 m |
| VI | Ca 3.5* | 4.23 m | 4.42 m | 3.50(2H, br.s)* | 3.23(2H, br.s) | 5.33 m |
| VII | 3.1-3.4* | 4.22 m | 4.37 m | (Me) | 3.25(2H, br.s)* | 5.33 m |
| VIII | Ca 3.5* | 4.08(t, 4) | 4.21 m | 3.48(2H, br.s)* | 3.73(2H, br.s) | 5.30 m |
| IX | 4.48<br>(q, 6, 8) | 4.07<br>(q, 3, 4) | 4.20(t, 4) | (Me) | 3.68(d, 12)<br>3.76(d, 12) | 5.30 m |
| X | Ca 3.5* | 4.16<br>(q, 3, 6.5) | 4.46(t, 5) | 3.5(2H, br.s)* | 9.43(1H, s) | 5.45 m |
| XI | (=O) | 4.17<br>(q, 2.5, 5.5) | 4.46(t, 4.5) | (Me) | 9.45(1H, s) | 5.50 m |
| XIV | 4.79<br>(q, 7.5, 8.5) | | 5.06<br>(q, 6, 12) | 3.75(d, 12.5)<br>3.85(d, 12.5) | 4.24(d, 11.5)<br>4.45(d, 11.5) | 5.50 m |
| XV | 3.0-3.25 | | 6.98<br>(q, 4, 7.5) | 3.40(d, 10)<br>3.70(d, 10) | | 5.55 m |

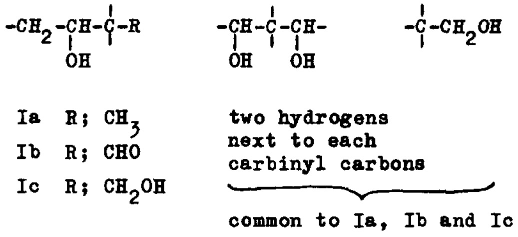
*Complex signals because of overlapping.

TABLE II. Methyl Signals in NMR Spectra of Camelliagenin Derivatives

| | $-\overset{|}{\underset{|}{C}}-C\underline{H}_3$ | | | | | | | $C\underline{H}_3CO_2-$ | $\begin{matrix}C\underline{H}_3\\C\underline{H}_3\end{matrix}>C<\begin{matrix}O-\\O-\end{matrix}$ |
|---|---|---|---|---|---|---|---|---|---|
| Compds | 23, 24, 25 | | | 26, 29, 30 | | | 27 | | |
| Ib | | 0.94 | 0.94 | 0.94 | 0.99 | 1.07 | 1.27 | | |
| II | 0.86 | 0.86 | 0.97 | 0.92 | 0.97 | 1.03 | 1.31 | 1.98 2.03(2)*<br>2.08 | |
| III | | 0.84 | 1.01 | 0.93 | 0.97 | 1.03 | 1.31 | 1.99 2.02 2.03<br>2.04 2.09 | |
| IV | | 0.85 | 1.01 | 0.93 | 0.93 | 1.03 | 1.44 | 2.04(3) 2.06 | |
| VI | | 1.00 | 1.05 | 0.89 | 0.94 | 1.07 | 1.30 | | 1.40(2) 1.45(2) |
| VII | 0.78 | 0.94 | 0.99 | 0.89 | 0.94 | 1.07 | 1.28 | | 1.40 1.45 |
| VIII | | 0.99 | 1.05 | 0.89 | 0.94 | 1.05 | 1.28 | 2.04 | 1.39-1.41(4) |
| IX | 0.86 | 0.86 | 0.96 | 0.90 | 0.94 | 1.05 | 1.26 | 2.05 2.07 | 1.38 1.41 |
| X | | 1.00 | 1.07 | 0.80 | 1.00 | 1.07 | 1.27 | | 1.42(2) 1.45(2) |
| XI | 1.05 | 1.05 | 1.10 | 0.85 | 1.00 | 1.05 | 1.26 | | 1.41 1.45 |
| XIV | | 0.86 | 1.02 | 0.94 | 1.02 | 1.06 | 1.25 | 2.01 2.02(2)<br>2.05 | |
| XV† | | 0.89 | 0.89 | 0.92 | 0.92 | 1.01 | 1.01 | | |

* Number of methyl groups overlapping is in parentheses. † Assignment is ambiguous.

hydrogens between VI and its acetate VIII, m.p. 196-197°, and between VII and its diacetate IX, m.p. 200-202°, disclosed that the 1,3-glycol unit in Ia consists of two secondary hydroxyl groups and that the additional 1,3-glycol system in Ic consists of primary and secondary hydroxyls (Table I). This was further confirmed by the chromic acid oxidation of the acetonides: VI gave an aldehyde X, m.p. 162.5-165°, $\nu$ 2718, 1712 cm$^{-1}$ and VII gave a keto-aldehyde XI, m.p. 212.5°, $\nu$ 2725, 1704 cm$^{-1}$. These findings, together with the splitting pattern of the carbinyl hydrogens and aldehydic protons revealed the substitution pattern A in the sapogenols.



$-CH_2-\underset{\underset{OH}{|}}{C}H-\overset{|}{\underset{|}{C}}-R$ $\quad$ $-\underset{\underset{OH}{|}}{C}H-\overset{|}{\underset{|}{C}}-\underset{\underset{OH}{|}}{C}H-$ $\quad$ $-\overset{|}{\underset{|}{C}}-CH_2OH$

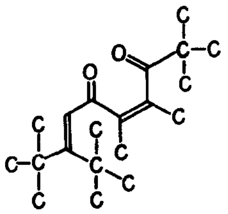
Ia R; $CH_3$
Ib R; CHO
Ic R; $CH_2OH$

two hydrogens next to each carbinyl carbons

common to Ia, Ib and Ic

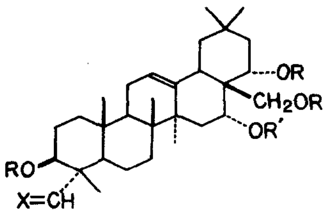
A

The carbon skeleton of these sapogenols was deduced in the following way. Selenium dioxide oxidation of VIII afforded, after reconversion to the diacetonide, the heteroannular diene diacetonide XII, $\lambda_{max}^{MeOH}$ 235 ($\varepsilon$ 17900 sh), 243 (27300), 252 (31300), 261 m$\mu$ (19600) (8). The NMR spectrum of XII exhibited two vinylic protons at 5.61 (q, J=11, 1.5) and 6.50 (q, J=11, 3), indicating the presence of a $-\underset{|}{C}H-CH=CH-\overset{|}{\underset{|}{C}}-$ grouping. Further selenium dioxide oxidation yielded the dienedione monoacetonide XIII, m.p. 266-268.5°, which displayed the expected spectral properties; $\lambda_{max}^{MeOH}$ 280 m$\mu$ ($\varepsilon$ 11400) (9), $\nu$ 3440, 1650, 1611 cm$^{-1}$, $\delta$ 5.93 (1H, sharp singlet). This clearly demonstrates the presence of grouping B in XIII and, when coupled with the presence of eight methyl groups (or their equivalent) attached to quaternary carbons (Table II) and of a vinylic proton signal of a typical shape for an olean-12-ene (Table I) in the NMR spectra of all derivatives listed in Table I, these observations led to the assignment of olean-12-ene as the carbon skeleton of the sapogenins.

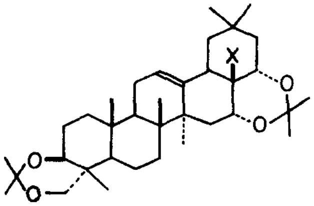


B

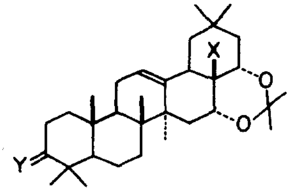
One of the secondary hydroxyl groups is considered to be at the 3β-position, because i) the carbinyl proton signal appearing at ca 3.2 in VII is shifted to 4.48 by acetylation (to IX) in accord with the behaviour of 3β-hydroxyolean-12-enes and their acetates, ii) in the derivatives of Ia, such as II, VII, IX and XI, signals assignable to 23-, 24- and 25-methyls always appear at the same field as other 3β-hydroxyolean-12-enes (10) and iii) these methyl signals change their chemical shifts regularly on going from the hydroxyl (VII) to



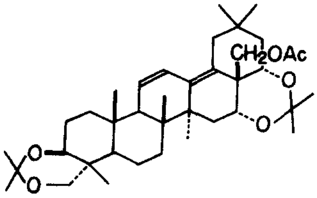
Ia R=R'=H X=$H_2$
Ib R=R'=H X=O
Ic R=R'=H X=H,OH
II R=R'=Ac X=$H_2$
III R=R'=Ac X=H,OAc
IV R=Ac R'=H X=H,OAc



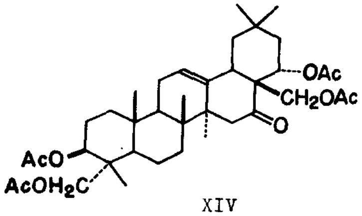
VI X=$CH_2OH$
VIII X=$CH_2OAc$
X X=CHO



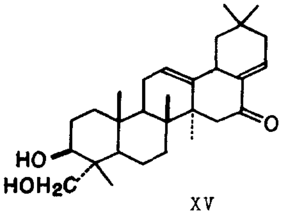
VII X=$CH_2OH$ Y= $<^{OH}_{H}$
IX X=$CH_2OAc$ Y= $<^{OAc}_{H}$
XI X=CHO Y=O



XII



XIV



XV

the acetoxyl compound (IX) (0.08, 0.02 and -0.13 ppm shifts) and from (IX) to the corresponding ketone (XI) (0.19, 0.09, 0.24 ppm shifts) (Table II) (11).

One of the primary hydroxyls in Ic, which is absent in Ia and aldehydic in Ib, forms an acetonide and a cyclic carbonate in each case with the 3β-hydroxyl group and must therefore be located at either the 23- or the 24-position. In fact, the 3α-H signal in III is deshielded compared with that in II (Table I). The equatorial nature of the hydroxymethyl group is revealed by the fact that i) the aldehydic proton of Ib appears at 9.39 ppm (12) and that ii) the methylene protons in the $-CH_2-OAc$ in III and IV appear as either a broad singlet or closely situated AB pattern (13) (this was also observed in XIV and XV described below). Thus the primary hydroxyl was placed at the 23-position while the two secondary and a primary hydroxyl groups, all of which are present in the three sapogenols Ia, Ib and Ic, remain to be located.

In the NMR spectrum of the tetraacetate IV, the carbinyl proton attached to the remaining hydroxyl group appears as a broad singlet, suggesting that the hydroxyl is axial. The ketone XIV, m.p. 231-231.5°, $\nu$ 1740 $cm^{-1}$, RD $[\Phi]^{trough}_{326}$ -7160, $[\Phi]^{peak}_{270}$ +8320, obtained by a $CrO_3$ oxidation of IV was subjected to an alkaline hydrolysis which afforded the norketone XV, m.p. 202-206°, $\lambda^{MeOH}_{max}$ 244 mμ ($\varepsilon$ 7500), $\nu$ 3310, 1692, 1626 $cm^{-1}$ (strong), $\delta$ 6.98 (1H, q, 7.5, 4), $\delta^{Benzene}$ 6.97 (1H, m). These spectral properties are indicative of a cisoid $\alpha\beta$-unsaturated carbonyl grouping with $\alpha$ and $\beta$-trans substituents (14). Furthermore, thermal decomposition (at 230°) of Ia, VI and VII liberates formaldehyde (dimedone) and the residue from the reaction of VI was reconverted to acetonide to give a triene with a trisubstituted heteroannular diene system, $C_{32}H_{48}O_2$, m.p. 147-149°, $\lambda^{MeOH}_{max}$ 238 mμ ($\varepsilon$ 11830), $\delta$ 5.38, 5.92 (J=10, AB), 5.4-5.7 (complex 2H). These results, in addition to the presence of the "1,3-diaxial" relationship (15) between the methyl group appearing at lowest field (assignable to the 27-methyl) and the hydroxyl group in IV (OH (IV)→OAc (III): $\delta_{Me}$, 1.44→1.31; OH (IV)→=O (XIV): $\delta_{Me}$, 1.44→1.25), enable us to place the hydroxyl groups at the 16$\alpha$-, 22- and 28-positions. The configuration of the 22-hydroxyl is revealed to be equatorial from the formation of acetonides and the coupling constant of the C-22 hydrogen signal in the NMR spectra of IV and XIV (Table I) (16).

The above evidence leads to assignment of structures Ia, Ib, and Ic for the camelliagenins A, B,and C, respectively.

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## References and Footnotes

1) M. Ishidate and K. Takamura, Yakugaku Zasshi (J. Pharm. Soc. Japan), **73**, 347 (1953) and the papers cited therein.

2) Idem., ibid., **74**, 641 (1954). K. Takamura, ibid., **74**, 645 (1954). Also see, J. Simonsen and W.C.J. Ross, The Terpenes, Vol. 4, p. 455, Cambridge Univ. Press, Cambridge (1957).

3) There has been a paper on the thin-layer chromatography of triterpenes in which the Rf values of three camellia sapogenols are listed. T. Murakami, H. Itokawa, F. Uzuki and N. Sawada, Chem. Pharm. Bull., **13**, 1346 (1965).

4) The results of elemental analyses agree with the formulation for all compounds described in this paper. $[\alpha]_D$s refer to ethanol solution. IR spectra were taken in KBr.

5) Ia and Ic have been shown to be identical (Rf value in TLC and IR spectrum) with Murakami's camellia sapogenols I and III, respectively. We are indebted to Dr. Itokawa, Tokyo College of Pharmacy, for the identification. Dr. Itokawa and his coworkers (3)

have agreed (cf. the following paper) that the names camelliagenin A, B, and C be used in place of camellia sapogenols I, II, and III in order to avoid unnecessary confusion in future.

6) NMR spectral data are listed in the Table I (for lower-field signals) and II (for methyl signals). Spectra were measured at 60 Mc (and 100 Mc in some cases) for deuterochloroform solutions. Chemical shifts are expressed in p.p.m. from internal tetramethylsilane. Coupling constants in c/s are given in parentheses together with signal multiplicities abbreviated as s (for singlet), br.s (for broad singlet), t (for triplet), and q (for quartet).

7) J.L. Hales, J.I. Jones and W. Kynaston, *J. Chem. Soc.*, 618 (1957), L. Hough and J.E. Priddle, *ibid.*, 581 (1961) and the papers cited therein.

8) L. Ruzicka, A. Grob and F. Vander Sluys-Veer, *Helv. Chim. Acta*, **22**, 767, 788 (1939).

9) L. Ruzicka and O. Jeger, *ibid.*, **24**, 1236 (1941).

10) B. Tursch, R. Savoir and G. Chiurdoglu, *Bull. Soc. Chim. Belges*, **75**, 107 (1966).

11) Positive values denote downfield shift. Cf. J.M. Lehn and G. Ourisson, *Bull. soc. chim. France*, 1137 (1962). S. Itô, M. Kodama and H. Hikino, unpublished data.

12) T.J. King and J.P. Yardley, *J. Chem. Soc.*, 4308 (1961).

13) A. Gandemer, J. Polonsky and E. Wenkert, *Bull. soc. chim. France*, 407 (1963).

14) For NMR solvent shift, see Y. Fujise and S. Itô, *Chem. Pharm. Bull.*, **14**, 797 (1966).

15) Y. Kawazoe, Y. Sato, M. Natsume, H. Hasegawa, T. Okamoto and K. Tsuda, *ibid.*, **10**, 338 (1962). R.F. Zürcher, *Helv. Chim. Acta*, **44**, 1380 (1961), **46**, 2054 (1963).

16) Although an equatorial disposition of this hydroxyl group is concluded for the acetates, the smaller coupling constants of the 22β-hydrogen in the acetonides, IX, X, and XI (Table I) suggest that the ring E has been deformed in such a way that the 22α-hydroxyl became α-axial and parallel to the 16α-hydroxyl. The absence of any intramolecular hydrogen bonding in VI and VII confirmed this assumption. Inspection of scale models indicates that the E ring in the acetonides is a twisted boat form and the 22β-hydrogen bisects the H-C-H angle of the 21-methylene.