Tetrahedron Letters No.27, pp. 2577-2580, 1967. Pergamon Press Ltd. Printed in Great Britain.

ON GENUINE SAPOGENINS OF HORSE CHESTNUT SAPONINS BY MEANS OF SOIL BACTERIAL HYDROLYSIS AND A NEW MINOR SAPOGENIN: 16-DESOXY-BARRINGTOGENOL C

Itiro Yosioka, Kanako Imai, and Isao Kitagawa

Faculty of Pharmaceutical Sciences, Osaka University

Toyonaka, Osaka, Japan.

(Received 25 April 1967)

Three pentacarbocyclic triterpenoid sapogenins aescigenin (I), protoaescigenin (II), and barringtogenol C (III) (=aescinidin) have hitherto been isolated from European horse chestnut[1] (Aesculus hippocastanum L., seeds) and, besides, tigloyl (or angeloyl) and acetyl derivatives of protoaescigenin have also been isolated by Kuhn and Loew from the same plant source[2]. As reported in the previous paper[3], we have been working on the sapogenin constituent of Japanese horse chestnut (A. turbinata Blume, seeds) and have elucidated the similarity of sapogenin compositions of both chestnuts and, in addition, isolated a new minor sapogenin tentatively designated Rx which is now disclosed to be 16-desoxy-barringtogenol C (IV) in this communication. Very recently, in connection with the studies on theasapogenols[4], we have proposed to revise the chemical structures of aforementioned horse chestnut sapogenins to I, II, and III respectively[5] as shown in Chart 1.

On the other hand, the problems on genuineness of some sapogenins, especially of the acid sensitive ones have been chased for these years by several research groups[1b,6]. In pursuit of genuine sapogenins, we have been developing a new enzymatic hydrolysis method of saponin by means of soil bacterial cultivation and were successfully able to obtain presenegenin[7], an acid-labile genuine sapogenin of Senega root[6a], in a good yield.

Protoaescigenin, the major sapogenin of horse chestnut, was initially approved to be genuine form of aescigenin[1a,b]. It was however mostly based on the speculative standpoint, because the former was always accompanied by the latter even under very mild acidic hydrolysis of saponin[1b]. In addition, at the same time, Tschesche and co-workers have proposed the structure of its mother saponin, aescin[8] having aescigenin in its sapogenin part. Therefore, it seems worthwhile to clarify the genuine sapogenin of horse chestnut in this connection. In this paper, we wish to describe the results using

soil bacterial hydrolysis method on Japanese horse chestnut saponins, which reveals protoaescigenin, barringtogenol C, and 16-desoxy-barringtogenol C being the genuine sapogenins, and moreover in which no indication was obtained concerning to the genuineness of aescigenin.

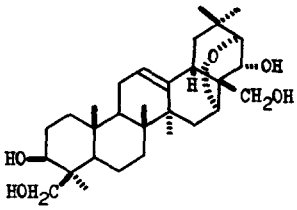
Thus, ether extraction of the total culture broth prepared by stationary cultivation (for 17 days, at 31°C) on saponin containing medium (3g. horse chestnut saponin in 1 liter synthetic medium) using the selected soil bacterial stam[9] as described previously[7], furnished 540 mg. of the extract, whose thin layer chromatogram (TLC) was as shown in Fig. 1. The components giving spots denoted by a, b, c', d, and e were isolated by repeated preparative TLC. As the compounds obtained from the fraction e appeared to be acylated sapogenins as obtained by Kuhn and Loew[2], they were next treated with KOH-MeOH yielding spots a, b, and d on TLC. The combined pure fractions corresponding to a, b, and d (weighed 40, 12, and 4 mg.) were identified with protoaescigenin, barringtogenol C, and 16-desoxy-barringtogenol C (the structure will be discussed below)(by TLC, IR), thus supporting their genuineness. Although the minor fraction c' having similar Rf values to c (aescigenin) could not be separated to their components, none of them was identical with aescigenin on TLC (using four different kinds of solvent systems). Furthermore, repeated experiments starting from different soil samples also gave the similar results, thus concluding that horse chestnut saponins should be formulated possessing protoaescigenin, barringtogenol C and 16-desoxy-barringtogenol C as their sapogenin moieties.

The compound previously named Rx[3], $C_{30}H_{50}O_4$, mp. 288-290.5°, $[\alpha]_D$ + 50° (c, 0.4 in EtOH), IR(KBr): 3378, 1630 cm^{-1}, afforded a tetraacetate (V), $C_{38}H_{58}O_8$, mp. 225-226°, $[\alpha]_D$ + 50° (c, 0.8 in $CHCl_3$) on treatment with acetic anhydride and pyridine. The nuclear magnetic resonance spectrum of V quite resembles to that of tetra-O-acetylbarringtogenol C (VI) except that V lacks the signal ascribable to a proton attached to C_{16}(Table 1.) Consequently, the structure of Rx is reasonably

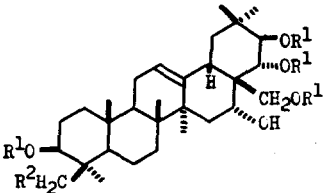
Table 1. (τ values in $CDCl_3$, at 60 Mc.)

	V	VI[4a]
$>C_{(3)}\underline{H}$-OAc	5.49 (t.-like)	5.45 (t.-like)
$>C_{(16)}\underline{H}$-OH	——	5.77 (m.)
$=C_{(12)}\underline{H}$	4.70 (m.)	4.60 (m.)
$-\overset{\vert}{C}_{(17)}-C\underline{H}_2$-OAc	6.16 (s)	6.30 (s)
$\underline{HC}_{(22)}$-OAc	5.06, 4.71 (*AB q. J=10 cps.)	4.54, 4.40 (AB q. J=10 cps.)
$\underline{HC}_{(21)}$-OAc		
$-\overset{\vert}{C}-$		

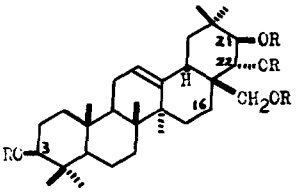
*Diamagnetically shifted probably due to the absence of deshielding effect caused by C_{16}-oxygen function.



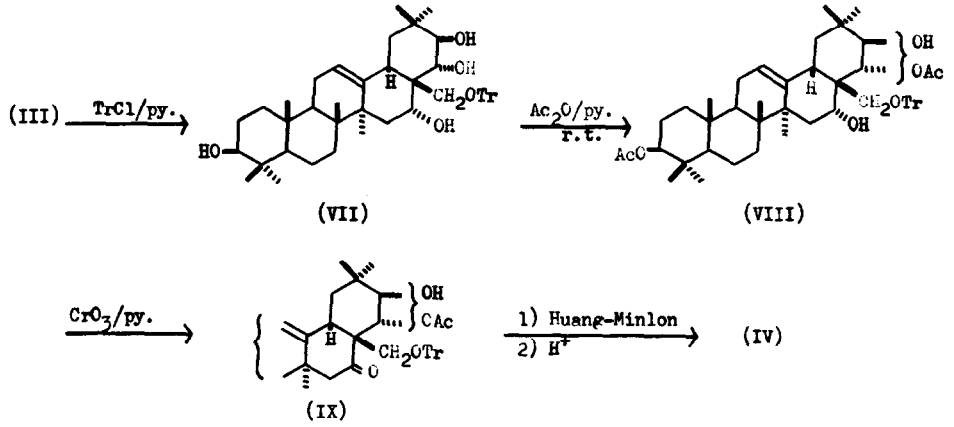
(I) aescigenin



(II) R^1=H, R^2=OH
protoaescigenin
(III) R^1=R^2=H
barringtogenol C
(=aescinidin)
(VI) R^1=Ac, R^2=H



(IV) R=H
16-desoxy-barringtogenol C
(V) R=Ac



(III) $\xrightarrow{TrCl/py.}$ (VII) $\xrightarrow[r.t.]{Ac_2O/py.}$ (VIII)

(VII) $\xrightarrow{CrO_3/py.}$ (IX) $\xrightarrow[2)\ H^+]{1)\ Huang\text{-}Minlon}$ (IV)

Chart 1.

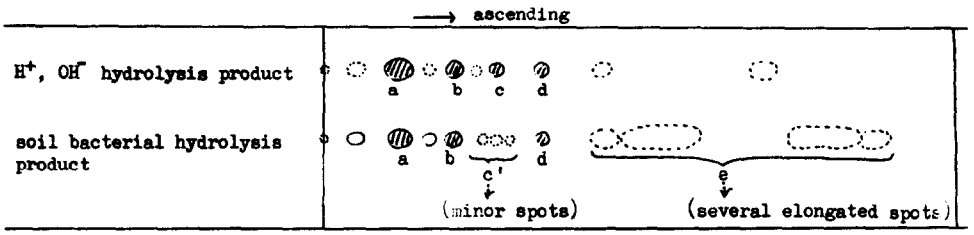


Fig. 1. TLC ($CHCl_3$: MeOH= 8:1 , Silica gel G) of the hydrolysis products obtained by chemical procedure (upper) and soil bacterial method (lower).
a= protoaescigenin (II), b= barringtogenol C (III),
c= aescigenin (I), d= 16-desoxy-barringtogenol C (IV)

expressed by 3β,21β,22α,28-tetrahydroxy-olean-12-ene (= 16-desoxy-barringtogenol C) (IV), which was verified by the reaction sequence as shown in Chart 1. The monotritylether of barringtogenol C (VII), yielded a diacetate (VIII), which on selective oxidation by CrO_3-pyridine was derived to a diacetyl-monoketone (IX). The ketone was then subjected to Huang-Minlon reduction followed by an acid treatment giving 16-desoxy-barringtogenol C which was proved to be identical with Rx by mixed mp., IR, and TLC.

The compounds described with chemical formulae gave satisfactory analytical data.

References

1)
a) C.Cainelli, A.Melera, D.Arigoni, O.Jeger: Helv.Chim.Acta, 40 2390 (1957).
b) R.Kuhn, and I.Loew: Liebig's Ann., 669 183 (1963).
c) R.Tschesche, G.Wulff: Tetrahedron Letters, 1965 1569.
2) R.Kuhn, I.Loew: Tetrahedron, 22 1899 (1966).
3) I.Yosioka, K.Imai, I.Kitagawa: Chem.Pharm.Bull. (Tokyo), 15 135 (1967).
4)
a) I.Yosioka, T.Nishimura, A.Matsuda, I.Kitagawa: Tetrahedron Letters, 1966 5973, 5979.
b) I.Yosioka, A.Matsuda, T.Nishimura, I.Kitagawa: Chem. & Ind., 1966 2202.
5) I.Yosioka, T.Nishimura, A.Matsuda, K.Imai, I.Kitagawa: Tetrahedron Letters, 1967 637.
6)
a) On Senega sapogenin: J.J.Dugan, P.de Mayo: Can.J.Chem., 43 2033 (1965).
Y.Shimizu, S.W.Pelletier: J.Am.Chem.Soc., 88 1544 (1966).
b) On Cyclamen sapogenin: R.Tschesche, F.Inchaurrondo, G.Wulff: Liebig's Ann., 680 107 (1964).
c) On Ginseng sapogenin: S.Shibata, O.Tanaka, T.Ando, M.Sado, S.Tsushima, T.Ohsawa: Chem.Pharm.Bull. (Tokyo), 14 595 (1966).
7) I.Yosioka, M.Fujio, M.Osamura, I.Kitagawa: Tetrahedron Letters, 1966 6303.
8) R.Tschesche, U.Axen, G.Snatzke: Liebig's Ann., 669 171 (1963).
9) The identification of soil bacterial stam used here is in progress.