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Components of Cortex Condurango

Tschesche and his co-workers studied the components of *Marsdenia cundurango* REICH. (Asclepiadaceae family), and described the isolation and structure of kondurangogenin-A[1], -C[2], and kondurangoglycoside-A, -A_1 and -C, -C_1.[3] We independently studied the components of cortex condurango commercially obtained in Japan. Dried and pulverized material was extracted with $CHCl_3$ and usual work-up for the isolation of glycoside in Asclepiadaceae family plants[4] gave a crude glycoside mixture, which was submitted to mild acid hydrolysis. After extraction with ether, aqueous layer gave a sugar mixture containing 2,6-dideoxy sugar, cymarose and oleandrose, detected by paper partition chromatography ($CHCl_3$/$HCONH_2$ system). Ether layer afforded an aglycone mixture, which was further hydrolyzed with 5% methanolic KOH. This reaction mixture was separated into acid and neutral portions and extracted with BuOH. Acid portion gave cinnamic acid, mp 131—132°, confirmed by direct comparison with an authentic sample. Neutral portion was submitted to chromatography over alumina, and gave sarcostin[5] (I), drevogenin-D[6] (II), dihydrodrevogenin-D[6] (III), and a new pregnane-type compound, for which the name marsdenin (IV) is proposed.

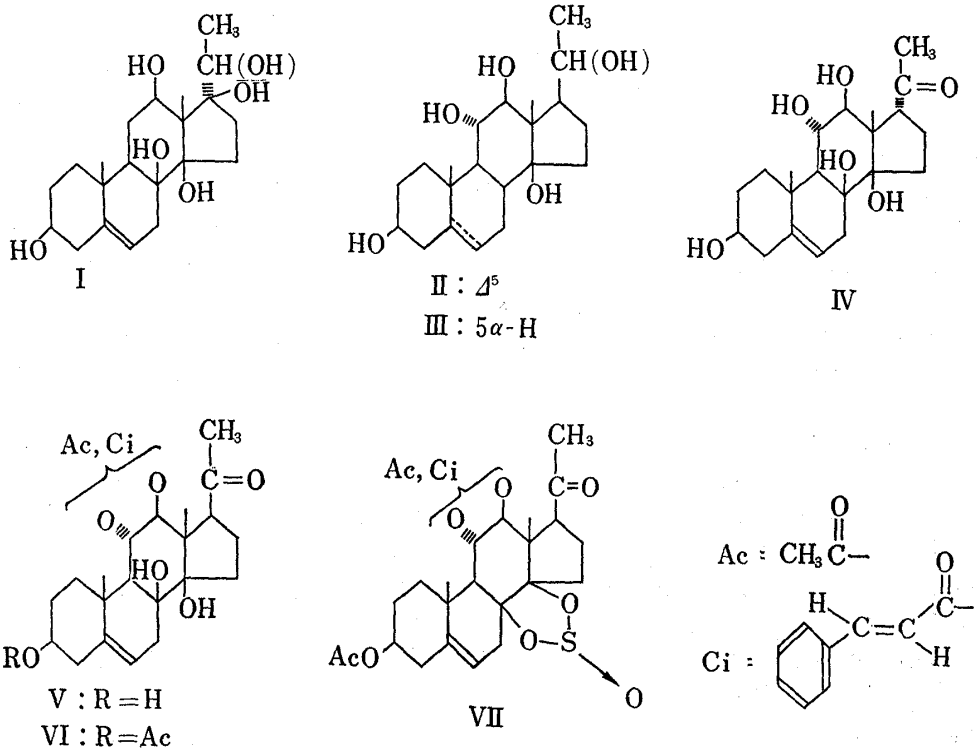

Chart 1

Marsdenin (IV), $C_{21}H_{32}O_6$ (M^+ 380), mp 260—264°, $[\alpha]_{589}$ −16.0° (c=0.22, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3400, 1680, NMR τ (in pyridine): 8.24 (3H, singlet), 8.08 (3H, singlet), 7.57 (3H,

1) R. Tschesche, P. Welzel, and G. Snatzke, *Tetrahedron*, **21**, 1777 (1965); R. Tschesche, P. Welzel, and H.W. Fehlhaber, *ibid*, **21**, 1797 (1965).
2) R. Tschesche, H. Kohl, and P. Welzel, *Tetrahedron*, **23**, 1461 (1967).
3) R. Tschesche and H. Kohl, *Tetrahedron*, **24**, 4359 (1968).
4) H. Mitsuhashi and T. Nomura, *Chem. Pharm. Bull.* (Tokyo), **13**, 274 (1965).
5) Y. Shimizu and H. Mitsuhashi, *Tetrahedron*, **24**, 4143 (1968).
6) H.H. Sauer, Ek. Weiss and T. Reichstein, *Helv. Chim. Acta*, **48**, 857 (1966); *Idem, ibid.*, **49**, 1655 (1967).

singlet), 6.09 (1H, doublet, J=11 cps), and 5.41 (1H, triplet, J=11 cps), and UV, end absorption at 210 mμ. It is deduced that marsdenin (IV) has a pregnane skeleton possessing C-20 oxo group and one double bond. The mass spectrum exhibits the ions m/e 380(M^+), 362(M^+–H_2O), 344(M^+–$2H_2O$), 329(M^+–$2H_2O$–$CH_3^\cdot$), 326(M^+–$3H_2O$), 311(M^+–$3H_2O$–$CH_3^\cdot$), 283(M^+–$3H_2O$–$CH_3CO^\cdot$). The ions m/e 224, 206, 163, 138, 120, and 105 appear to be derived from a retro-Diels-Alder reaction of the 5—6 double bond[7] in the B-ring as illustrated in Chart 2. Hence, only one hydroxyl group is in the ring A and B, and the hydroxyl group may be at C-3β from the biogenetic analogy. Consumption of 2 molar equivalent of $Pb(OAc)_4$ suggests the presence of two α-glycol groups in marsdenin (IV). One is at C-11α and C-12β from its coupling constant (J=11 cps, axial-axial coupling). Highly deshielded signals of τ 8.24 and 8.08 suggest the presence of C-8β hydroxyl group, with 1—3 diaxial relation to both C-18 and C-19 methyl groups. The other α-glycol, therefore, may be at C-8β and C-14β position from the biogenetic analogy to other natural polyhydroxy-pregnanes.

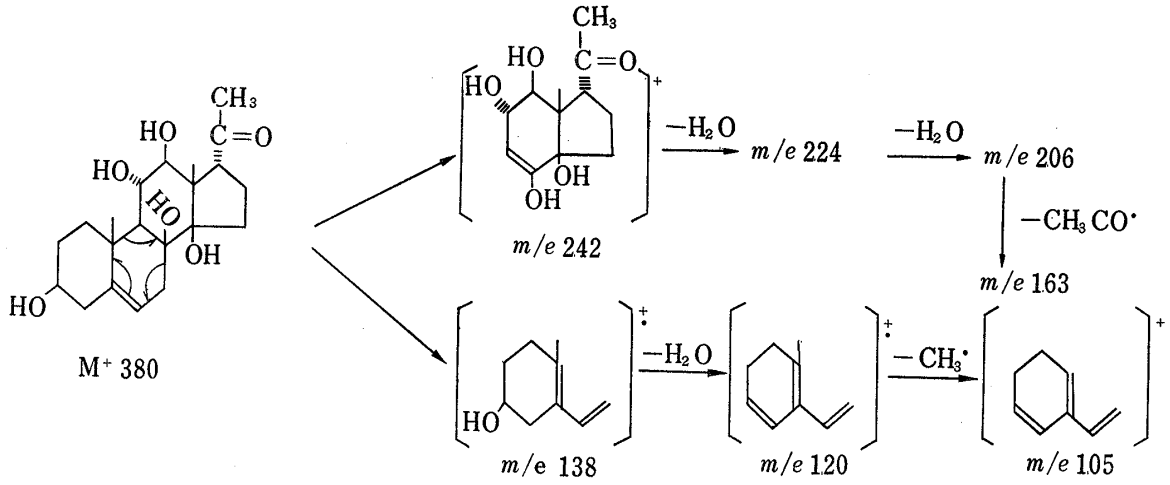

Chart 2

On the other hand, the aglycone mixture was submitted to alumina column chromatography and silica gel thin-layer chromatography, and five ester-type aglycones were isolated, each of these esters being pure on thin-layer chromatogram. One of them,[8] ester-C (V), vitreous, M^+ 552, $[\alpha]_{589}$ +95.2° (c=0.21, MeOH), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3450, 1755, 1715, 1710, 1638, 1275, 1250, and 1175, UV λ_{max}^{EtOH} mμ (log ε): 218 (4.19), 224 (4.16), and 280 (4.34), ORD positive Cotton effect, exhibits in its NMR spectrum (in $CDCl_3$) the signals at τ: 8.71(3H, singlet), 8.66 (3H, singlet), 8.03 (3H, singlet), 7,77 (3H, singlet), 6.80 (1H, multiplet), 6.40(1H, multiplet, half width *ca.* 15 cps), 5.00 (1H, doublet, J=11 cps), 4.62 (1H, multiplet), 4.14 (1H, triplet, J=11 cps), 3.56 (1H, doublet, J=16 cps), 2.57 (5H, multiplet), and 2.27 (1H, doublet, J=16 cps). These data show that ester-C has an acetyl and a cinnamoyl ester moiety. Alkali hydrolysis of ester-C gave a crystalline mass as a neutral portion which was identified with marsdenin (IV), and cinnamic acid as acidic portion. Ester-C gave a vitreous monoacetate, $[\alpha]_D$ +93.3° (c=0.388, MeOH), which showed infrared bands at 3600 and 3400 cm^{-1} ($CHCl_3$). Treatment of the monoacetate (VI) with $SOCl_2$ in pyridine afforded a five-membered cyclic sulfite[9] (VII), mp 230—234°; *Anal.* Calcd. for $C_{34}H_{40}O_{10}S$: C, 63.73; H, 6.29. Found: C, 63.53; H, 6.32, $[\alpha]_D$ +170.6° (c=0.212, MeOH), which shows no hydroxyl band but shows a five-membered cyclic sulfite at 1215 cm^{-1} [10] in its infrared spectrum (Nujol). These experi-

7) B. Kapur, H. Allgeier, and T. Reichstein, *Helv. Chim. Acta*, **50**, 2147 (1967).
8) The structure of other esters will be described in another paper.
9) A. Von Wartburf and J. Renz, *Helv. Chim. Acta*, **42**, 1620 (1959): R. Tschesche and G. Marwede, *Tetrahedron Letters*, **1967**, 1359.
10) P.B.D. de la Mer, W. Klyne, D.J. Millen, J.G. Pritchard, and D. Watson, *J. Chem. Soc.*, **1956**, 1813.

mental evidences exhibit the presence of α-glycol at 8β and 14β. C-17 side chain of marsdenin (IV) may be β-H oriented from its ORD data[11] (negative Cotton effect, a=−88.1). Finally, we propose the structure of (IV) for marsdenin and of (V) for ester-C as shown in Chart 1.

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Multi-Order Microdiffusion Analysis

The microdiffusion analysis was established by Conway and his co-workers and up to this time, the method has estimated only one component released from a sample. However, this is anyhow a special case and in general there occour two or more volatile components. If the previous method is named one order microdiffusion analysis, the following method should be called a multi-order microdiffusion analysis. Namely, the components may be estimated in a unit operation whereby to release more than two volatile components simultaneously and to absorb them in two or more absorbents respectively. The technique is generally named a "multi-order microdiffusion analysis."

In this paper, two order microdiffusion analysis was applied to two experiments; to a simultaneous determination of ethanol and carbon dioxide in alcohol fermentation, and to that of ammonia and carbon dioxide in hydrolysis of urea by urease. Theoretical speculation will be discussed in the future but from the experimental data the followings were revealed:

Apparatus: Conway-Ishizaka's semi-micro unit (the outer chamber: inner diameter 90 mm, inner height 17 mm; the inner chamber: outer diameter 65 mm, inner diameter 60 mm, inner height 8.5 mm) contains two larger cups (inner diameter 22.1 mm, inner height 12 mm) for the use of absorbents and if necessary, such as in the case of alcohol fermentation, one additional smaller cup (inner diameter 12.5 mm, inner height 12 mm) was used instead of outer chamber for the use of fermentation medium, because the culture solution decreased considerably by the strong dehydration ability of acidic oxidative absorbent.

Procedure: It was carried out according to the general operation of unit, but taking the sample solution in a smaller cup in the case of alcohol fermentation. Incubation and diffusion temperature: 30—37°.

Reagents: For the alcohol fermentation: (1) 1.0 ml of 1 N KOH for the absorbent of CO_2. (2) 1.0 ml of 2 N $K_2Cr_2O_7$ in 10 N H_2SO_4 for the absorbent of C_2H_5OH. (3) 0.5 ml of Henneberg's culture medium (4% saccharose) for the analytic test and 0.5 ml of *Saccharomyces cerevisiae* var. Sake suspension (*ca.* 10^6—10^{10} cells/ml). For the blind test, it was treated without saccharose. (4) As the inhibitor of fermentation, 0.5 ml of 15% T.C.A was used.