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Detection of Proto-type Compounds of Diosgenin- and Other Spirostanol-Glycosides

In the course of studies on the distribution of allantoin[1] and of diosbulbin B and related furanoid diterpenoids[2] in some *Dioscorea* plants, a number of compounds were detected on thin-layer chromatograms which showed red with Ehrlich reagent[3] (E-reag.) and were different from allantoin and diosbulbin B which gave yellow and reddish purple spots, respectively, with the same reagent. These compounds were also distinguished by the color (yellow) developed with anisaldehyde reagent[3] (A-reag.) from allantoin (greenish yellow) and diosbulbin B (purple). Diosgenin, yonogenin, tokorogenin and their glycosides which are known[4] to occur in the genus *Dioscorea* and other spirostanols (tigogenin, gitogenin, sarsasapogenin (and their glycosides), hecogenin, kryptogenin, pennogenin, bethogenin) showed the same color (yellow) with A-reag., but all were negative with E-reag. β-Sitosterol and digitoxin which gave purple and dark blue spots with A-reag., respectively, were also negative in Ehrlich reaction. Accordingly these unknown substances were supposed to be a new series of compounds.

A fraction obtained from methanol extractives of the fresh rhizomes of *Dioscorea tenuipes* Franch. et Sav. revealed five red spots with E-reag. on thin-layer and it was chromatographed on a silicic acid column (solvent, chloroform–methanol 4:1) to give two homogeneous compounds, white powder, mp 172—174° (decomp.), $[\alpha]_D^{15}$ −13.5° (c=0.52, pyridine) (peracetate mp 96—99°) (tentatively designated as compound Sa), and white powder, mp 185—189° (decomp.), $[\alpha]_D^{15}$ −69.2° (c=0.87, pyridine) (peracetate mp 122—126°) (Sd). When the fresh rhizomes of *D. gracillima* Miq. were extracted with methanol at room temperature the extractives contained four compounds of red Ehrlich reaction which were separated on a silica gel column (solvent, chloroform–methanol–water 67:33:10) to yield three of them in a homogeneous state: white powder, mp 142—180° (decomp.) (designated as PPD); colorless needles (recrystallized from methanol), mp 200—202° (decomp.), $[\alpha]_D^{18}$ −98.0° (c=1.0, pyridine) (peracetate mp 125—127°, $[\alpha]_D^{18}$ −45.1° (c=0.98, chloroform)) (MPD); colorless needles (from water), mp 225—230° (decomp.), $[\alpha]_D^{18}$ −69.7° (c=0.85, pyridine) (peracetate mp 123—124°, $[\alpha]_D^{18}$ −47.4° (c=0.73, chloroform)) (PD).

Except for Sa,[5] the four compounds Sd, PPD, MPD and PD were hydrolyzed with acid equally to give diosgenin, D-glucose and L-rhamnose, while with almond emulsin or commercial β-glucosidase Sd yielded gracillin[6] accompanied by a small amount of dioscin,[6] PPD gave a prosapogenin[7] of dioscin, and MPD and PD afforded dioscin, in all cases along with D-glucose. The above results indicate that they are the parent saponins, having additional mole(s) of D-glucose, of gracillin, a prosapogenin of dioscin, and dioscin, respectively. However, since diosgenin and its glycosides are negative to E-reag., these positive compounds do not

1) M. Hutoh, S. Kiyosawa, T. Morishima, and M. Konoshima, presented at the 87th Annual Meeting of Pharmaceutical Society of Japan, Kyoto, April 1967.
2) T. Kawasaki, presented at the 11th Pacific Science Congress, Tokyo, August 1966; T. Komori and T. Kawasaki, presented at the 88th Annual Meeting of Pharmaceutical Society of Japan, Tokyo, April 1968 ; T. Kawasaki, T. Komori, and S. Setoguchi, *Chem. Pharm. Bull*, (Tokyo), submitted.
3) E. Stahl, "Dünnschicht-Chromatographie," Springer-Verlag, Berlin, 1962, pp. 498, 503.
4) R. Hegnauer, "Chemotaxonomie der Pflanzen," Band II, Birkhäuser Verlag, Basel, 1963, p. 133; T. Kawasaki and T. Yamauchi, *Yakugaku Zasshi*, **83**, 757 (1963).
5) On acid hydrolysis of Sa tokorogenin, arabinose and rhamnose were provided.
6) T.Kawasaki and T. Yamauchi, *Chem. Pharm. Bull.* (Tokyo), **10**, 703 (1962).
7) T. Tsukamoto, T. Kawasaki, and T. Yamauchi, *Chem. Pharm. Bull.* (Tokyo), **4**, 35 (1956) ; T. Kawasaki and T. Yamauchi, *ibid.*, **16**, 1070 (1968).

seem to have diosgenin as their aglycones. The infrared spectra which showed no characteristic absorptions[8] of spiroketal side chain and the facile conversion to diosgenin or its glycoside suggest that they have the aglycones of furostane structure. It was supported by the fact that synthetic pseudodiosgenin-3, 26-diacetate, -3-O-glycoside peracetates, pseudotigogenin-3,26-diacetate, -3-O-glycoside peracetates and 22-methoxy-5α-furostane-3β,26-diol diacetate showed the same colors as those of new compounds with E- and A-reags. Furthermore, as reported in the forthcoming communication,[9] kikubasaponin from *D. septemloba* Thumb. which was regarded[6] as a diosgenin tetraglycoside shows the red Ehrlich reaction and is in fact correctly formulated as 22-methoxyfurost-5-ene-3β,26-diol 3,26-bis-O-glycoside, and the corresponding 22-hydroxy compound, a major constituent of the fresh rhizomes of the same plants, is also positive to E-reag. The structures of PD and MPD have also been established[10] as furost-5-ene-3β,22,26-triol 3,26-bis-O-glycoside and the 22-O-methyl derivative, respectively. Therefore Sd, PPD and other new compounds of red Ehrlich reaction are also considered to have the furostanol structure (probably 22-hydroxy (or alkoxy) 3,26-bis-O-glycoside).

Recently Schreiber and Ripperger[11] have reported a new steroid saponin jurubine from *Solanum paniculatum* L. and Tschesche, Lüdke and Wulff[12] have isolated, besides parillin, the second main saponin sarsaparilloside from *Smilax aristolochiaefolia* Mill. They have been respectively assigned the structures 25S-3β-amino-5α-furostane-22α,26-diol 26-O-glycoside[11] and 25S-5β-furostane-3β,22α,26-triol 3,26-bis-O-glycoside[12] which represent the first furostanol glycosides and support the Marker's suggestion[13] that the spirostanols might be the artefacts produced by acid hydrolysis of the glycosides having ring-E or -F opened aglycone. According to Tschesche and his co-workers[12] sarsaparilloside is a parent glycoside of parillin and the furostanol glycosides occur also in *Convallaria majalis, Digitalis purpurea, Dig. lanata* and *Avena sativa*.

Since the furostanol glycosides are considered[12,14] to be the proto-type compounds originally existing in the plants, from which the spirostanol glycosides are formed secondarily, they are expected to occur widely in the plants which are known to have steroid saponins. Some *Dioscorea* plants have now been found to contain this kind of glycosides.

The red Ehrlich reaction seems to be characteristic to the furost-20(22)-ene or 22-hydroxy (or alkoxy)-furostane derivatives among the plant steroids, and the thin-layer chromatography using E- and A-reags. for color development may be of value as a simple and convenient method for the preliminary examination[15] of the proto-type compounds of spirostanol glycosides (steroid saponins).

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9) T. Kawasaki and K. Miyahara, *Chem. Pharm. Bull.* (Tokyo), in preparation.
10) T. Kawasaki, T. Komori, T. Nohara, and I. Hosokawa, *Chem. Pharm. Bull.* (Tokyo), in preparation.
11) K. Schreiber and H. Ripperger, *Tetrahedron Letters*, **1966**, 5997 ; H.Ripperger, H. Budzikiewicz, and K. Shreiber, *Chem. Ber.*, **100**, 1725 (1967).
12) R. Tschesche, G. Lüdke, and G. Wulff, *Tetrahedron Letters*, **1967**, 2785.
13) R.E. Marker and J. Lopez, *J. Am. Chem. Soc.*, **69**, 2389 (1947).
14) It has often been observed that the contents of dioscin and gracillin in the fresh rhizomes are much smaller than those in the stored materials. Examination by means of thin-layer chromatography indicated the predominant existence of the proto-type compounds of red Ehrlich reaction in the fresh rhizomes.
15) Examination of forty kinds of Liliaceae plants suggested the presence of a number of furostanol glycosides (K. Tanaka, S. Kiyosawa, and M. Hutoh, presented at the 88th Annual Meeting of Pharmaceutical Society of Japan, Tokyo, April 1968).

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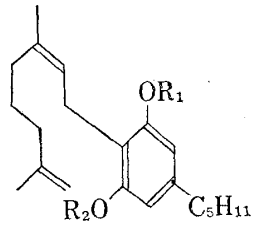
Cannabigerol Monomethyl Ether, a New Component of Hemp[1)]

In this communication, we wish to report the isolation of cannabigerol monomethyl ether (II), a new marihuana component, from the domestic hemp "Minamioshihara No. 1."[2)]

Benzene percolate of the leaves was treated as previously described,[1)] the acid mixture being heated with toluene for 7 hr to afford the phenol mixture, which was subjected to column chromatography on silica gel with benzene as eluant. A small amount of pale yellow syrupy substance (A) was obtained prior to the fraction of tetrahydrocannabinol (THC) and its purity was examined by thin-layer chromatography (TLC) and gas liquid chromatography (GLC).[3)] A, $C_{22}H_{34}O_2$, UV λ_{max}^{EtOH} mμ (ε): 230 (11200), 272 (1150), 280 (1090), gave yellow color with benzidine reagent[4)] but no staining with Beam's reagent[5)] which is available for diol such as cannabidiol (CBD) or cannabigerol (CBG) (I). The NMR spectrum showed the singlet peak due to $-O-CH_3$ protons at δ 3.78, besides identical feature with that of CBG.[6)] In the mass spectrum of A, the molecular ion peak at *m/e* 330 and other fragment ion peaks of M–56, M–69, M–83, M–85 and M–123 correspond to those of CBG[7)] with shift of 14 mass unit, indicating that A is CBG monomethyl ether.



I : $R_1=R_2=H$
II : $R_1=CH_3$, $R_2=H$
III : $R_1=R_2=CH_3$

On the methylation of CBG with diazomethane in methanol at 0°, followed by TLC separation, II was obtained together with CBG dimethyl ether (III) in a ratio of *ca.* 1:1. The physical constants and the spectra of the former substance were identical with those of A.

Since any phenols have not been detected in the fresh leaves of the hemp, A should be preserved in phenol carboxylic acid form as in the case of other marihuana components in nature.[8)] Cannabigerolic acid monomethyl ether, genuine substance of CBG monomethyl ether, is probably formed by the methylation of cannbigerolic acid, and this step suggests the third route which, as well as the pathway for tetrahydrocannabinolic acid and for cannabichromenic acid, arise from cannabigerolic acid.

1) This forms Part III of "Cannabis." Part II: Y. Shoyama, T. Fujita, T. Yamauchi, and I. Nishioka, *Chem. Pharm. Bull.* (Tokyo), **16**, 1157 (1968).
2) The presence of this substance was observed thin-layer- and gas liquid-chromatographically in all strains of the hemps cultivated in this university.
3) GLC was run in the same way as previously described.[1)]
4) J.E. Koch and W. Krieg, *Chemiker Ztg.*, **62**, 140 (1938); CBG: yellow, CBD: orange yellow, THC: orange, cannabinol: reddish orange.
5) F. Korte and H. Sieper, *J. Chromatog.*, **13**, 90 (1964).
6) R. Mechoulam and Y. Gaoni, *Tetrahedron*, **21**, 1223 (1965).
7) H. Budzikiewicz, R.T. Alpin, D.A. Lightner, C. Djerassi, R. Mechoulam, and Y. Gaoni, *Tetrahedron*, **21**, 1881 (1965).
8) T. Yamauchi, Y. Shoyama, H. Aramaki, T. Azuma, and I. Nishioka, *Chem. Pharm. Bull.* (Tokyo), **15**, 1075 (1967).