with sulfuric acid in methanol[6] afforded 16-oxotestosterone.[7]

Further studies on the hydrolysis of the esters possessing other acyloxyl groups and other aspects of this enzyme reaction will be reported in detail in a later publication.

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[6] W. S. Johnson, B. Gastambide, R. Pappo : J. Am. Chem. Soc., **79**, 1991(1957).
[7] A. S. Meyer, M. C. Lindberg : *Ibid.*, **76**, 3033(1954); W. J. Adams, *et al.* : J. Chem. Soc., **1956**, 297.

UDC 547.918:582.938

On the Isolation and Structure of Penupogenin

The isolation of an aglycone, cynanchogenin, from the root of *Cynanchum caudatum* Max.[1] was reported previously and the structure (A) was given for it.[2,3] In this communication, isolation of another aglycone, penupogenin, from the same root and its structure are described.

The aglycone mixture, obtained by the hydrolysis of the crude glycoside from the root,[1] was chromatographed over alumina column and the mixture separated into two fractions. The first mainly consisted of cynanchogenin. The second, which gave green Lieberman-Burchard reaction, was rechromatographed over alumina column. The eluate with 1% MeOH-$CHCl_3$ gave needles from ether-petroleum ether. Further recrystallizations from ether gave white needles, m.p. 145~150°, and this product was named penupogenin[*1] (II).

Penupogenin shows green color with Lieberman-Burchard reaction, orange→violet with conc. H_2SO_4, pink→greyish blue with $SbCl_3$, and negative Keller-Kiliani reaction. UV λ_{max}^{EtOH} 279 mμ (ε 22,000); IR λ_{max}^{KBr} cm^{-1} : 3400 (OH), 1690 (C=O conj.), 1630 (C=C conj.), 1580, 1600 (aromatic). These data strongly suggest that penupogenin is a cinnamoyl ester.

Hydrolysis of penupogenin gave an acid substance (III), m.p. 136°, and a neutral substance (IV), m.p. 260°/150° (from MeOH). The acid substance (III) gave only one spot on the chromatogram and was identified as cinnamic acid by the mixed m.p. (IV) gave much the same color reactions as (II) and was expected to have the formula $C_{21}H_{34}O_6$ (*Anal.* Calcd. : C, 65.94; H, 8.96. Found : C, 65.66; H, 7.78). Acetylation of (IV) afforded a triacetate, m.p. 205°, $C_{27}H_{40}O_9$ (*Anal.* Calcd. : C, 63.76; H, 7.59. Found : C, 63.45; H, 7.58).

In 1939, Cornforth, *et al.*[4] isolated sarcostin, $C_{21}H_{34}O_6$, from an Australian Asclepiadaceae plant, *Sarcostemma australe* R. Br. and Reichstein, *et al.*[6] also isolated the same

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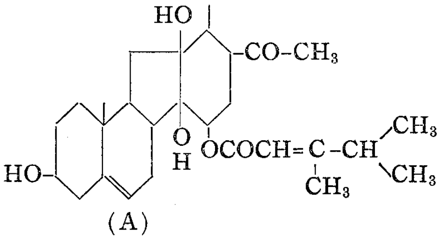
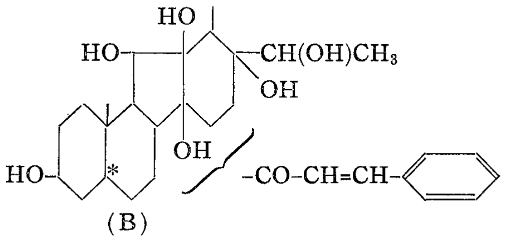
[*1] "Penup" is one of the Ainu names for *Cynanchum caudatum* Max.
[1] H. Mitsuhashi, Y. Shimizu : This Bulletin, **8**, 313(1960).
[2] *Idem* : *Ibid.*, **8**, 318(1960).
[3] *Idem* : *Ibid.*, **7**, 749, 949(1959).
[4] J. W. Cornforth, *et al.* : J. Chem. Soc., **1939**, 737; **1940**, 1443.
[5] T. Reichstein, *et al.* : Helv. Chim. Acta, **42**, 1014(1959).
[6] J. W. Cornforth : Chem. & Ind. (London), **1959**, 602.

substance from an African Asclepiadaceae plant, *Pachycarpus lineolatus*. (IV) was very similar to sarcostin and the fact was confirmed by admixtures of sarcostin and its triacetate with the present samples. From the tentative structure of sarcostin reported by Cornforth,[5] the structure of penupogenin would be cinnamoylsarcostin (B). Compounds which occur in the same plant usually have close resemblance in their structures, but the structures of the two compounds, deacylcynanchogenin and sarcostin, especially the locations of hydroxyl groups, are still open to question and further investigations are now in progress.


(A)


(B)

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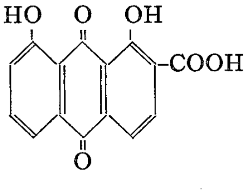
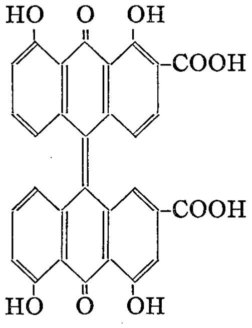
Structure of Rhein

Recently, Hörhammer, *et al.*[1,2] reported that rhein was converted quantitatively by treatment with acid into another substance which reverted again to rhein on alkaline treatment in the presence of air, and this observation led them to conclude that the previously described rhein (I)[3,4] must be "di-rhein" (II) and the compound obtained by the


(I)


(II)

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1) H. Wagner, I. Köhler : Naturwissenschaften, **44**, 260(1957); L. Hörhammer, H. Wagner, I. Köhler: *Ibid.*, **45**, 389(1958).
2) L. Hörhammer, H. Wagner, I. Köhler : Arch. Pharm., **292**, 591(1959).
3) O. A. Oesterle, E. Tisza : Schweiz. Wochschr. Chem. Pharm., **46**, 701(1908); O. Fischer, F. Falco, H. Gross : J. prakt. Chem., [2] **83**, 208(1911); O. Hesse : Pharm. J., [4] **1**, 325(1895); A. Tschirch, K. Heuberger : Arch. Pharm., **240**, 596, 610(1902); A. Stoll, B. Becker, W. Kussmaul : Helv. Chim. Acta, **32**, 1892(1949).
4) O. A. Oesterle : Arch. Pharm., **241**, 604(1903).

acid treatment is the "monorhein" having the structure recognized so far for rhein (I).

Since some doubts were felt in their report, the structure of rhein was reëxamined in this laboratory. This communication points out that the hypothesis of monorhein–dirhein by Hörhammer, *et al.* was derived from faulty reasonings, and proves that rhein certainly has the structure of (I) as has long been accepted and the proposed "monorhein" is rhein methyl ester in reality.

The German workers obtained rhein, m.p. 316~319°, i.e. their "di-rhein," from *Rheum palmatum*, aloin, and sennoside. A solution of 200 mg. of this compound dissolved in potassium hydroxide solution and methanol, was refluxed with an excess of hydrochloric acid for two hours. Sublimation of the reaction products afforded "monorhein" (120 mg.), m.p. 175°, and "monorheinanthranol" (20 mg.).

Since rhein has been known to be insusceptible to boiling hydrochloric acid, "monorhein" was deemed to be rhein methyl ester, and the above experiment of Hörhammer, *et al.* was repeated with the preparation of rhein, m.p. 318~319° (decomp.) (Fig. 1; IR 1701 cm^{-1} COOH), isolated in this laboratory from the fresh rhizome of *Rheum coreanum* Nakai. Following exactly the conditions reported, rhein (100 mg.) was treated with hydrochloric acid and methanol, and a mixture of two substances was obtained which gave Rf values of 0.32 and 0.84 on the paper chromatogram (solvent system : butanol saturated with water, ascending method). If the components are "monorhein" and "monorheinanthranol," the mixture should naturally be extractable with sodium hydrogencarbonate solution. The present experiment showed, however, that the mixture is largely insoluble in the hydrogencarbonate solution and, from this insoluble portion, there was obtained a compound (60 mg.) melting at 174° (Rf 0.84) by recrystallization from chloroform and methanol. The product was found identical with rhein methyl ester described in the

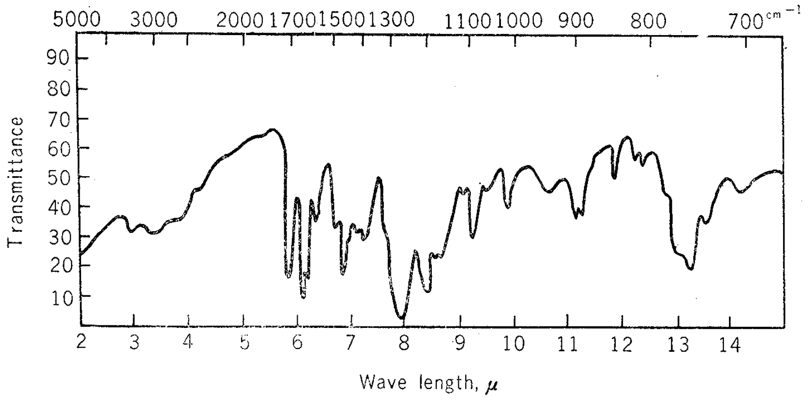

Fig. 1. Infrared Spectrum of Rhein (KBr)

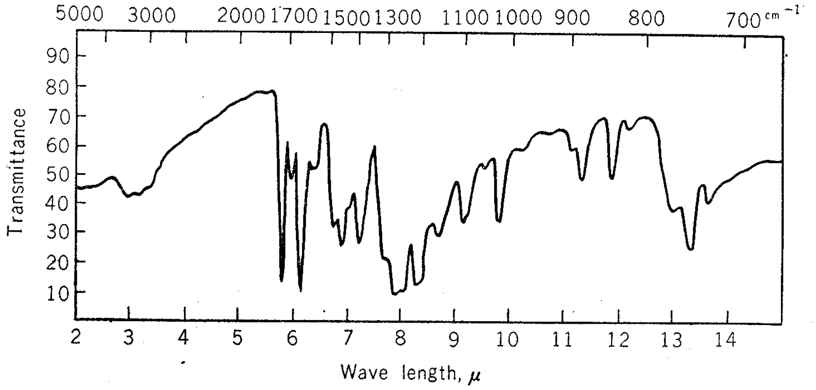

Fig. 2. Infrared Spectrum of Rhein Methyl Ester (KBr)

literature[5] and was confirmed by analysis, mixed melting point, and infrared spectrum to be the same as the rhein methyl ester, m.p. 175°, prepared from rhein by the action of diazomethane. Furthermore, the infrared spectrum (Fig. 2; 1733 cm^{-1} ester; 1684 cm^{-1} free CO) of this compound was completely superimposable with the spectrum of "monorhein."[2] These results constitute an irrefutable evidence of the identity of "monorhein" with rhein methyl ester. From the alkaline layer of the above extraction 20 mg. of rhein (Rf 0.32) was recovered, which was characterized by the melting point and infrared spectrum.

For the "monorhein–dirhein" hypothesis, following four grounds were given: 1) Conversion of rhein to monorhein and monorheinanthranol by oxidative and reductive cleavages, 2) formation of di-rhein by dimerization of monorhein, 3) absence, in the infrared spectrum of di-rhein, of a band corresponding to the non-chelated carbonyl group which is observed in that of monorhein, and 4) thermochromism. In view of the present conclusion, the above reasons clearly lose their meaning. Interconversion of "monorhein" and "di-rhein" obviously does not involve such complicated reactions as they had imagined, but is merely a process of esterification and hydrolysis.

The infrared spectral data are emphasized as the main ground for the above hypothesis. Apparent absence of a band corresponding to the non-chelated carbonyl group in the spectrum of rhein (Hörhammer's di-rhein) can be interpreted as being due to the overlapping of a neighboring strong band of the carboxyl, and appearance of a carbonyl band in the spectrum of rhein methyl ester (Hörhammer's monorhein) is well understood as owing to the shift of the neighboring carboxyl band to a higher frequency by esterification. It may also be more reasonable to attribute the absorption at 1733 cm^{-1} in the spectrum of rhein methyl ester to an ester group than to regard the band as due to carboxyl. Thermochromism[6] observed with "di-rhein" has been adduced as an evidence for the presence of an aromatic-substituted ethylene linkage. However, it is still a question whether to take this phenomenon as a decisive proof for the presence of the linkage or not.

The German workers further mentioned the biosynthesis of rhein and indicated the simultaneous occurrence of "monorhein" and "di-rhein" in the rhubarb extracts. It is evident from the present experiment that partial esterification may easily have occurred during their extraction process which involved a treatment with methanol and hydrochloric acid.

Table I.

Compound	Formula	m.p. (°C)	Analyses (%)							
			Calcd.				Found			
			C	H			C	H		
Rhein[3,4]	$C_{15}H_8O_6$	318~319	63.39	2.84			63.40	2.85		
Rhein methyl ester[5]	$C_{16}H_{10}O_6$	174	64.43	3.38	OCH_3	10.39	64.33	3.37	OCH_3	10.55
Rhein ethyl ester[5,7]	$C_{17}H_{12}O_6$	150~153	65.38	3.87			65.39	3.76		
Rhein diacetate[4,5,8]	$C_{19}H_{12}O_8$	250	61.96	3.29			61.80	3.27		
Rhein diacetyl ethyl ester[5]	$C_{21}H_{16}O_8$	157~159	63.63	4.07	OC_2H_5	11.35	63.45	3.97	OC_2H_5	11.43

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5) R. Robinson, J. L. Simonsen: J. Chem. Soc., **95**, 1085(1909).

6) G. Kortüm: Angew. Chem., **70**, 14(1958).

7) O. A. Oesterle, G. Riat: Arch. Pharm., **247**, 527(1909).

8) A. Tschirch, P. A. A. F. Eijken: Schweiz. Wochschr. Chem. Pharm., **42**, 549(1904); F. Tutin, H. W. B. Clewer: J. Chem. Soc., **99**, 946(1911).

On the combustion analysis, their samples of "monorhein" and "di-rhein" left a large amount of residue and consequently the results are inconsistent even with the calculated values of their own. To account for this, they affirmed that rhein might resist combustion and so the result of the elemental analysis is not worthy of serious consideration. Such difficulties in combustion of rhein have never been encountered by any other researchers,[3,4] including the present writers who all obtained the experimental values agreeing well with the values calculated for structure (I).

Data for rhein and its derivatives prepared in the present experiment are listed in Table I and all these data support the conclusion of the present study.

A full account of this investigation will be published elsewhere.

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