SELECTIVE CLEAVAGE OF GLUCURONIDE LINKAGE IN OLIGOGLYCOSIDE

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Selective cleavage of a specific glycoside linkage in oligoglycoside (*e.g.* saponin) is considered to be useful especially for structure elucidation of the carbohydrate portion in the oligoglycoside. This article summarizes the recent investigations on the selective cleavage methods for the glucuronide linkage in oligoglycoside (glucuronide-saponin) which have been undertaken in the authors' laboratory.

1. Introduction
2. Photolysis
3. Lead Tetraacetate Degradation
4. Acetic Anhydride-Pyridine Degradation
5. Anodic Oxidation
6. Conclusion

1. Introduction

In the course of structural studies on oligoglycosides such as saponins, acid hydrolysis has been commonly used for cleavage of the glycoside linkage between the aglycone and the carbohydrate residue. It has been demonstrated, however, that the aglycone, especially the acid-labile one, sometimes suffers secondary alteration during the hydrolysis procedure, and this makes the structure elucidation of saponin rather complicated.

In order to avoid such undesirable side reactions, some devices have been made for cleavage of the glycoside linkage and for obtaining the genuine sapogenol (or aglycone): *e.g.* periodate oxidation[1] and soil bacterial hydrolysis.[2] However, these methods are disadvantageous for elucidating the structure of the oligosaccharide portion in oligoglycoside. Recently, enzymatic hydrolysis using the crude glycosidase mixture has been successfully applied in increasing number of the cases for structure elucidation of saponins.[3]

In this connection, any chemical method, by which a specific glycoside linkage in oligoglycoside is selectively cleaved, has been considered to be extremely useful, especially for structure elucidation of the carbohydrate portion of oligoglycoside. Although this kind of studies have been the interesting subjects of many investigations on polysaccharide,[4] no work has been reported in the field of saponin chemistry. For these years, we have been interested in exploiting a new chemical method which effects selective cleavage of a specific glycoside linkage in oligoglycoside.

In this review, we describe the outline of four selective cleavage methods for the glucuronide linkage in oligoglycoside (saponin) which have been recently developed in our laboratory. They are photolysis,[5] lead tetraacetate degradation,[6] acetic anhydride and pyridine degradation,[7] and

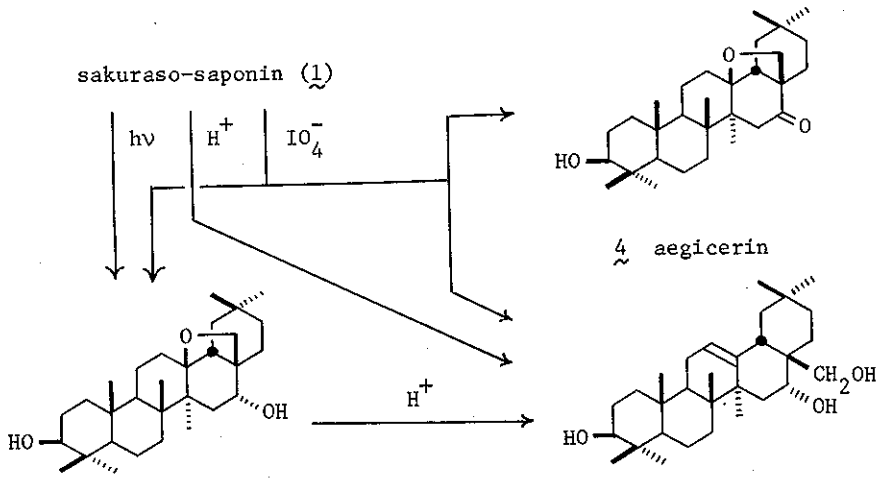
anodic oxidation.[8] By use of these methods, saponins, which possess a glucuronide moiety directly attached to the sapogenol (abbreviated as glucuronide-saponin hereafter), are readily decomposed to extricate their genuine sapogenols and to furnish, in most cases, the carbohydrate ingredients.

2. Photolysis[5]

Being suggested from the mass spectrometric behavior of oligoglycoside derivatives, it was initially anticipated that the glycoside linkage in oligoglycoside might be photochemically cleaved. After some preliminary examinations on the photochemical behavior of various oligoglycosides at hand, it has been found that the glucuronide linkage in oligoglycoside is readily cleaved by ultraviolet light irradiation.

For example, irradiation of sakuraso-saponin (1)[9] in methanol for 1 hr with a 500 W high pressure mercury lamp (equipped with a Vycor filter) liberates the genuine sapogenol, protoprimulagenin A (2), in a 74% yield. Acid hydrolysis of the same saponin, on the other hand, affords only primulagenin A (3) and the genuine sapogenol (2) is not obtained. Protoprimulagenin A (2) can be isolated in a lesser yield by repeated periodate oxidation of sakuraso-saponin (1), but formation of another minor artifact sapogenol (4, aegicerin), in addition to 3, is always accompanied.[10] Therefore, photolysis has been shown to be a convenient method for liberating the genuine sapogenol of sakuraso-saponin (1).

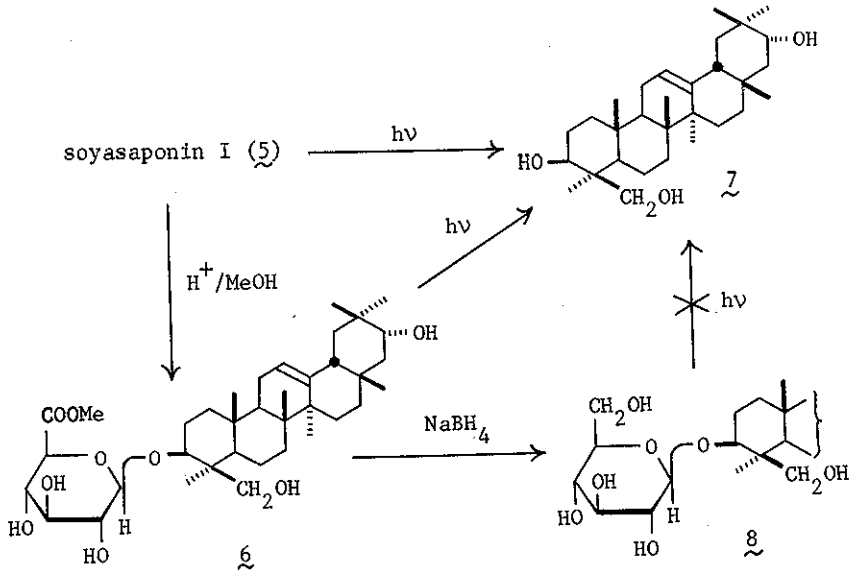
In order to clarify general applicability of the photochemical cleavage method, various saponins at hand have been examined. It has been found that following glucuronide-saponins readily liberate their genuine sapogenols upon ultraviolet light irradiation: *e.g.* kurinso-saponin from *Primula japonica*



4 aegicerin

2 protoprimulagenin A

3 primulagenin A



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6

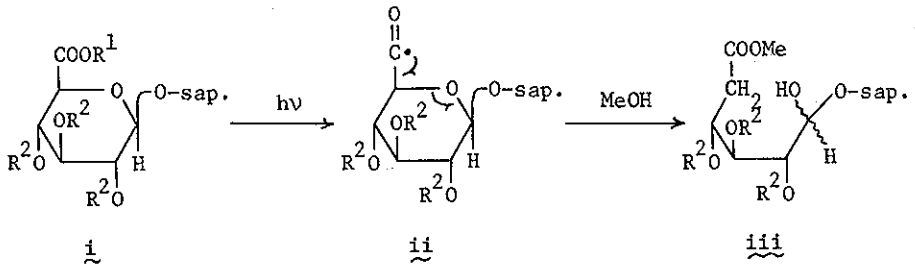
8

A. Gray (root),[10] soyasaponin I (5) from *Glycine max* Merrill (seeds),[11] desacyl-jegosaponin (28) from *Styrax japonica* Sieb. et Zucc. (pericarps),[12] desacyl-boninsaponin A (30) from *Schima mertensiana* Koidz. (bark),[13] desacyl derivatives of tea seeds saponin mixture from *Thea sinensis* L. (seeds),[14] and desacyl derivatives of horse-chestnuts saponin mixture from *Aesculus turbinata* Blume (seeds).[15]

The role of glucuronic acid moiety has been next investigated. Ultraviolet light irradiation of a prosapogenol methyl ester (6) liberates soyasapogenol B (7) in an excellent yield, while a glucoside (8), which is prepared by sodium borohydride reduction of 6, is unaffected under the same photolytic conditions. Consequently, it has become clear that cleavage of the glycoside linkage is initiated by a photochemical reaction of the carboxyl function in the glucuronide moiety. In combination with a fact that the photolysis does not proceed in an alkaline medium, a scheme I (i → ii → iii →) is suggested as a plausible reaction pathway for the photochemical cleavage of the glucuronide linkage.

On the other hand, in the case of chikusetsusaponin IV (9)[16] and V (10),[17] which are bisdesmosides having an additional ester-glucoside linkage along with a glucuronide bond, selective cleavage of the glucuronide linkage giving β-D-glucopyranosyl oleanolate (11)[2a] is effected.

As described above, ultraviolet light irradiation is a simple procedure for cleavage of the glucuronide linkage in glucuronide-saponin and for liberating the genuine sapogenol. This is the first example of the photolytic cleavage of glycoside linkage in oligoglycoside which contains a photo-sensitive function in the carbohydrate portion but no such a function in the aglycone part. Some related studies have been reported on the photolysis of aryl glycosides[18] and cellulose.[19]



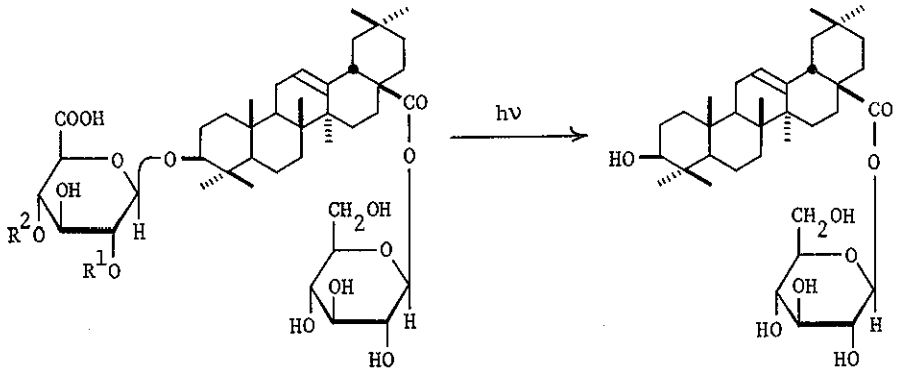
i ii iii

Scheme I : R^1= H or Me

R^2= H or carbohydrate residue

sap.= sapogenol

sapogenol



9 R^1= H, R^2= α-L-arabinofuranosyl (chikusetsusaponin IV)

10 R^1= β-D-glucopyranosyl, R^2= H (chikusetsusaponin V)

11

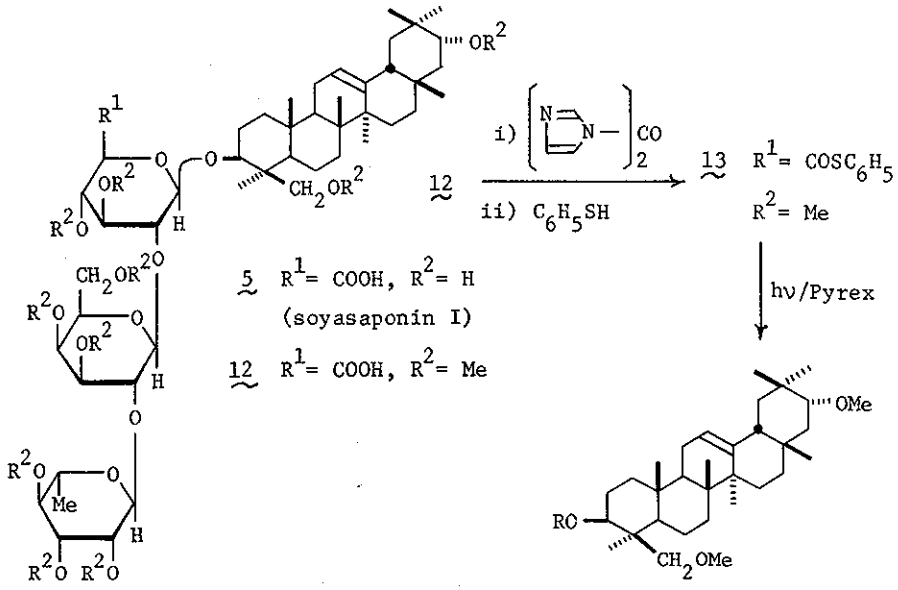
Since ultraviolet light transparent through a Vycor filter is required for the photolysis, application of the method is rather limited and seems to be unsuitable for glucuronide-saponin having a photo-sensitive sapogenol(*e.g.* glycyrrhizin). In addition, any product, which is derived from the carbohydrate portion, has not yet been isolated probably due to photochemical decomposition. Therefore, the studies have been developed to find out suitable modification of the carboxyl function in the glucuronic acid moiety of glucuronide-saponin (*e.g.* R^1=$COOCH_3$, CHO, or $COSC_6H_5$ in 12), in order to effect cleavage of the glucuronide linkage with longer wave-length light.

Among these, a phenylthio ester group (as in 13), which is readily prepared from a carboxyl group (as in 12) by treatment with N,N'-carbonyldiimidazole followed by addition of thiophenol, has been found so far to be a promising photosensitive group and to be cleaved by ultraviolet light irradiation through a Pyrex filter (giving 14), although the yield of 14 is unsatisfactory.[5d]

The photolytic method is yet a subject of further investigation. However, it should be mentioned here that photochemical cleavage of the glucuronide linkage is an excellent method in a sence, because hydrolysis of the glucuronide linkage usually requires stronger acid conditions as compared with hydrolysis of the ordinary neutral glycoside linkage. Furthermore, if the sapogenol portion is photo-stable as in sakuraso-saponin (1), the photolytic method is favorable for liberation of the genuine sapogenol such as protoprimulagenin A (3), which contains an acid-labile 13β,28-oxide moiety.

3. Lead Tetraacetate Degradation[6]

In the case of photolysis, a photochemical reaction of the carboxyl group in the glucuronic acid moiety functions as the initiating step leading to



5 R^1= COOH, R^2= H (soyasaponin I)

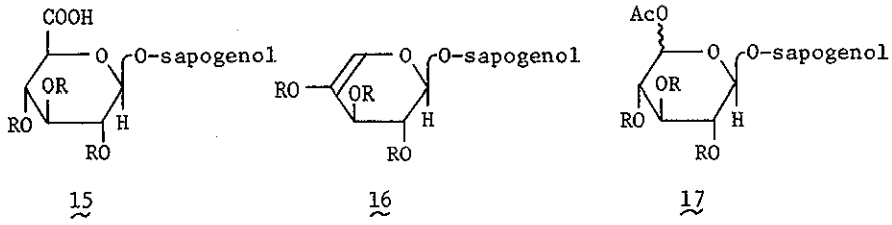
12 R^1= COOH, R^2= Me

12 i) ii) C_6H_5SH

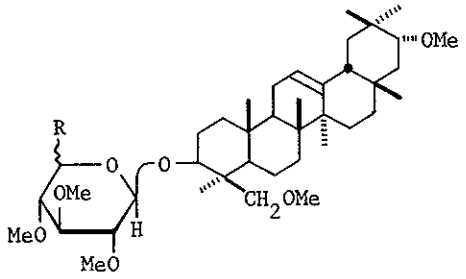
13 R^1= $COSC_6H_5$, R^2= Me

hν/Pyrex

14 R= H ; 14a R= Ac



15 16 17



18 R= β-COOH

19 R= α-OAc

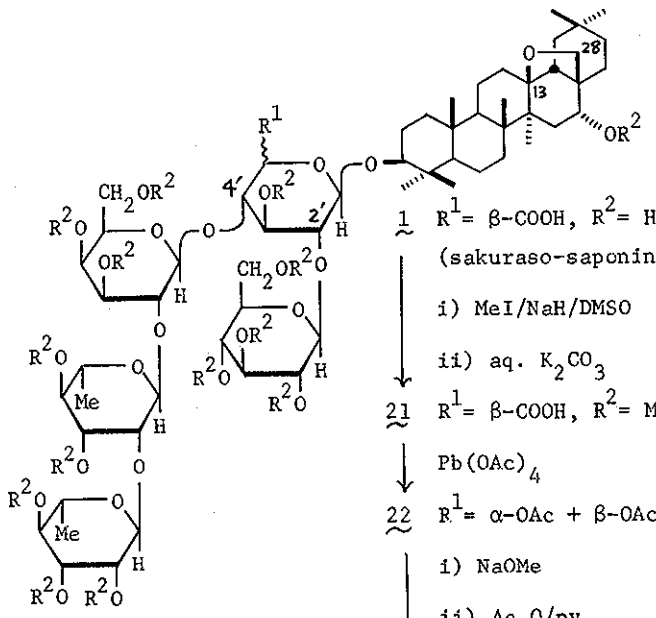
20 R= β-OAc

cleavage of the glucuronide linkage. Therefore, the similar type of reactions of the carboxyl group have been investigated. Although oxidative decarboxylation of a carboxyl group using lead tetraacetate has been known in various cases,[20] no work has been reported on the reaction between the carboxyl group of uronic acid and lead tetraacetate.

It was initially assumed that, when a glucopyranosiduronic acid (15, R= protecting group or carbohydrate residue) is treated with lead tetraacetate, an enol-type compound (16) and/or an acetate-type derivative (17) would be mainly formed and the resulting products would be readily decomposed either with mild acid or mild alkali treatment to furnish the sapogenol.

At the beginning, a glucuronide derivative (18), which is prepared from a prosapogenol (6) of soyasaponin I (5) and retains the free carboxyl group, has been subjected to lead tetraacetate oxidation. Treatment of 18 with lead tetraacetate in benzene under reflux for 1 hr yields two acetates (19, 45% and 20, 42%) in excellent yields. However, no product of type 16 is obtained. Subsequent treatment of both acetates (19, 20), respectively, with 0.1% sodium methoxide-methanol at room temperature for 30 min furnishes expected sapogenol (14) almost quantitatively.

Next, the method has been applied to a derivative (21) of sakurasosaponin (1). Reaction of 21 with lead tetraacetate yields a mixture of two epimeric acetates (22) in a 95% yield. Subsequent treatment of 22 with sodium methoxide-methanol followed by acetylation (acetic anhydride-pyridine, for facile separation of the products) furnishes following products: 23 (90%) from the sapogenol portion and a diene (24, 32%), two glucopyranosyl acetates (25 and 26, in a 70% combined yield), and a trisaccharide derivative (27, 92%) which are derived from the carbohydrate portion. The yields of all products are very high except 24 which is a quite unstable diene. The



1 R^1= β-COOH, R^2= H
(sakuraso-saponin)

i) MeI/NaH/DMSO

ii) aq. K_2CO_3

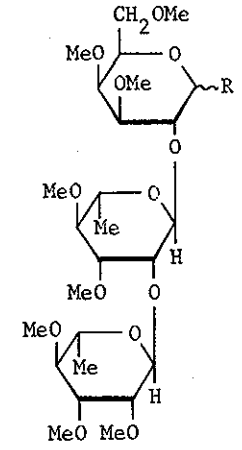
21 R^1= β-COOH, R^2= Me

$Pb(OAc)_4$

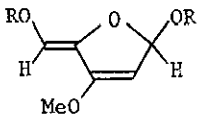
22 R^1= α-OAc + β-OAc, R^2= Me

i) NaOMe

ii) Ac_2O/py.

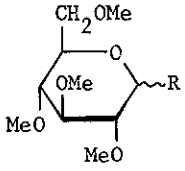


27 R= β-OAc + α-OAc



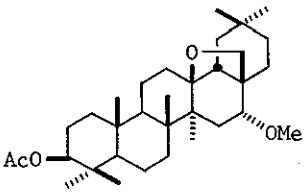
24 R= H

24a R= Ac



25 R= β-OAc

26 R= α-OAc



23

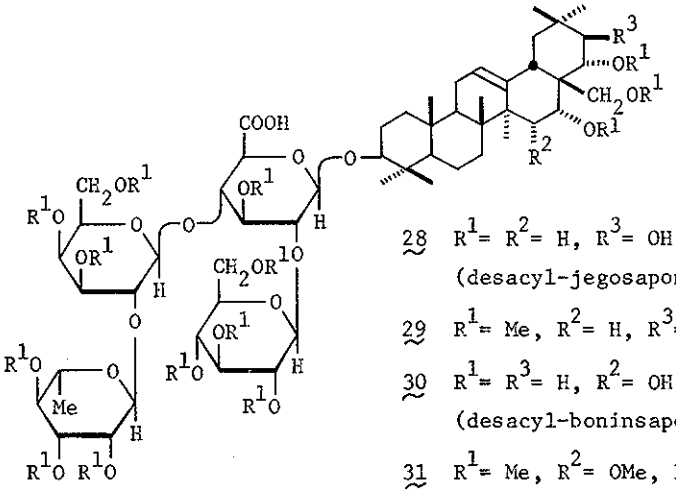
structure of 24 is substantiated by its physicochemical properties and by the similar analysis of its desacetyl derivative (24a).

As is apparent from the sequel, lead tetraacetate oxidation followed by alkali treatment is an excellent procedure for degradation of a permethylated derivative of glucuronide-saponin (preserving its free carboxyl function) and for liberation of its constituents in high yields. The residues at C-2' and C-4' in the glucuronic acid moiety are eliminated during the reaction sequence, and the method seems to be quite useful for structure elucidation of glucuronide-saponin. In addition, it is noteworthy that the acid-labile function in the sapogenol (*e.g.* the 13β,28-oxide moiety in 1) has been kept intact during the degradation procedure (as seen in 23).

In order to clarify the scope of the lead tetraacetate degradation method, some other glucuronide-saponins: *e.g.* soyasaponin I (5),[11] desacyl-jegosaponin (28),[12] and desacyl-boninsaponin A (30),[13] have been subjected to the method. Starting from carboxylic acid derivatives(12, 29, 31), expected ingredients (14a, 32, 33 from 12; 25, 26, 32, 33, 34 from 29; 25, 26, 32, 33, 35 from 31) are liberated in good yields, respectively, in addition to the diene (24) as a common product.

Based on these findings, the lead tetraacetate degradation method seems to be useful not only for structure elucidation of glucuronide-saponin, but also for selective cleavage of the glucuronide linkage in polysaccharide. Next, the behavior of methyl glycoside derivatives of D-glucuronic acid and D-galacturonic acid has been examined, since several kinds of uronide linkages other than β-glucuronide are known to occur in nature.

Treatment of methyl 2,3,4-tri-O-methyl-β-D-glucopyranosiduronic acid (36) with lead tetraacetate in refluxing benzene yields two major acetates (37, 42% and 38, 30%) and two minor ester-type disaccharides (39, 5% and 40, 3%). The

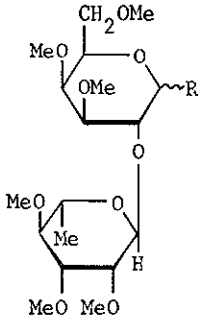


28 R^1= R^2= H, R^3= OH
(desacyl-jegosaponin)

29 R^1= Me, R^2= H, R^3= OMe

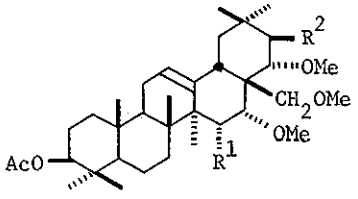
30 R^1= R^3= H, R^2= OH
(desacyl-boninsaponin A)

31 R^1= Me, R^2= OMe, R^3= H



32 R= β-OAc

33 R= α-OAc



34 R^1= H, R^2= OMe

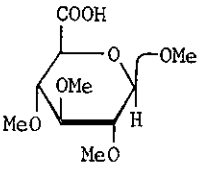
35 R^1= OMe, R^2= H

latter esters are probably formed by combination of an intermediary carbonium cation (iv)[21] with the starting compound (36). Analogous type of dimeric ester is not formed in the case of glucuronide-saponin presumably due to steric congestion at C-5' in the glucuronide moiety. Sodium methoxide-methanol treatment followed by acetylation of 37 and 38 furnishes the diene (24) in 69% and 74% yield, respectively. Here again, the substituents at C-2 and C-4 of 37 and 38 are readily eliminated during the conversion leading to 24. On similar methanolic alkaline treatment of 39 and 40, the methyl ester of 36 and the diene (24) are obtained in high yields.

Next, methyl 2,3,4-tri-O-methyl-α-D-glucopyranosiduronic acid (41) has been subjected to the degradation. In this case, two acetates (42, 43) are mainly formed, but no ester-type disaccharide is produced probably due to steric congestion at C-5. Alkaline treatment followed by acetylation of 42 and 43, gives 24 respectively, thus possible cleavage of the α-D-glucuronide linkage in oligoglycoside being demonstrated.

In the case of D-galacturonide linkage, the results are somewhat diverse. Treatment of methyl 2,3,4-tri-O-methyl-β-D-galactopyranosiduronic acid (44) with lead tetraacetate yields a 5α-acetate (45, 73%) as a single product. On subsequent treatment with sodium methoxide-methanol followed by acetylation, the acetate (45) is readily converted to the same diene (24) as from the above mentioned glucuronide derivatives. It has been shown therefore that the β-D-galacturonide linkage in oligoglycoside may be cleaved readily by the lead tetraacetate degradation method.

However, methyl 2,3,4-tri-O-methyl-α-D-galactopyranosiduronic acid (46) shows considerable resistance for lead tetraacetate oxidation probably due to steric congestion. Treatment of 46 with large excess of lead tetraacetate on prolonged heating (30 hr) in refluxing benzene yields a small amount of a



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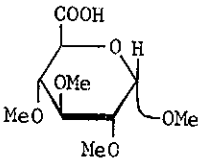
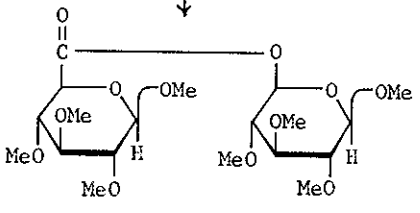


41

$Pb(OAc)_4$

$Pb(OAc)_4$



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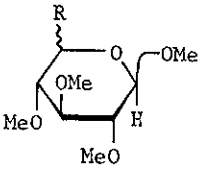
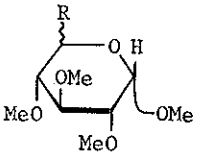


37 R= β-OAc

38 R= α-OAc



42 R= β-OAc

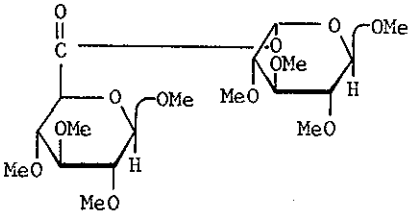
43 R= α-OAc

i) NaOMe

ii) Ac_2O/py.

i) NaOMe

ii) Ac_2O/py.



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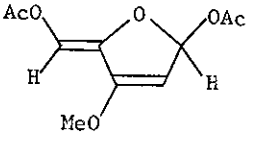

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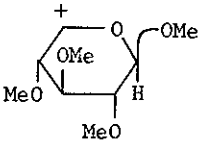
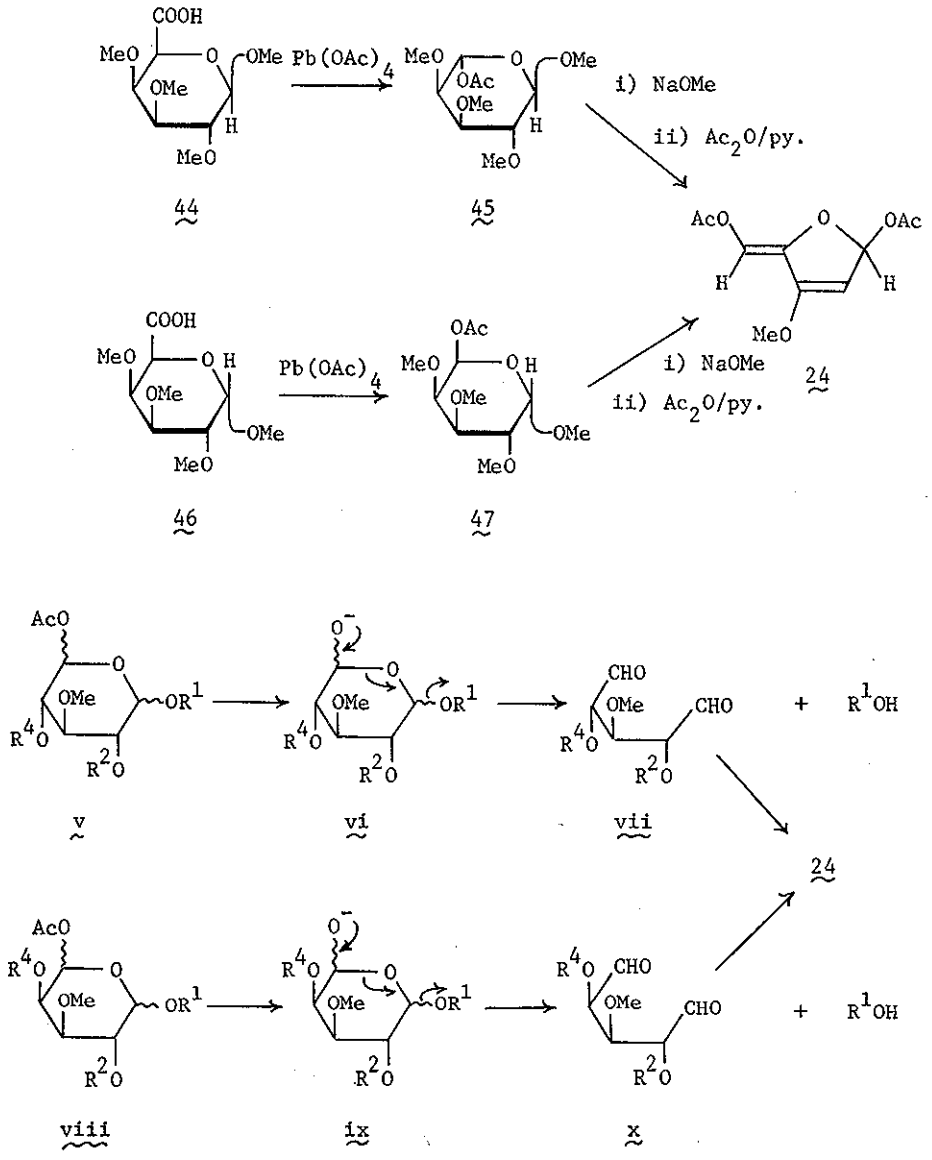


iv



Scheme II R^1= Me or sapogenol

R^2, R^4= Me or methylated carbohydrate residue

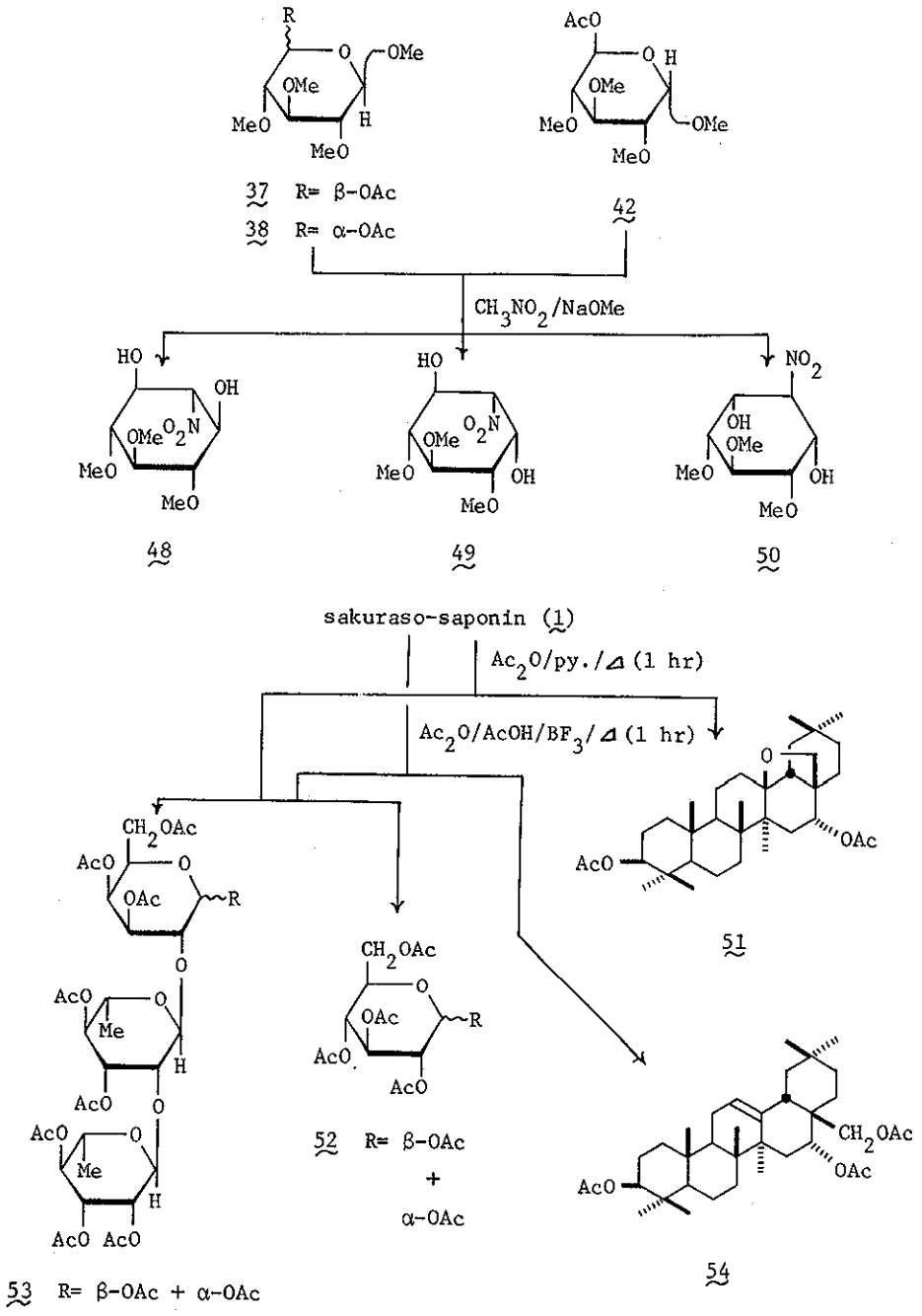
5β-acetate (47, 7%) with a recovery of most of the starting acid (46, 82%). The acetate (47) once formed is readily converted to the diene (24) as above in a high yield.

It has become clear so far that the lead tetraacetate degradation method is useful for degradation of the β- and α-D-glucuronide linkages and the β-D-galacturonide linkage. A more suitable cleavage method for the α-D-galacturonide linkage is under study in our laboratory. As for the reaction pathway, scheme II (v → vi → vii → 24 and viii → ix → x → 24) seems to be reasonable.

In order to define the reaction pathway, isolation of the dialdehyde intermediate (vii or x in scheme II) has been first attempted but without success. Finally, formation of the dialdehyde in the reaction medium has been substantiated by trapping it with nitromethane[22] under the alkaline conditions. Treatment of the acetate (37, 38, or 42) in nitromethane with 1N sodium methoxide-methanol at 4° for 12 hr yields three nitrocyclitols: 5-deoxy-1,2,3-tri-O-methyl-5-nitro-*scillo*-inositol (48), DL-1-deoxy-3,4,5-tri-O-methyl-1-nitro-*myo*-inositol(49), and 3-deoxy-1,5,6-tri-O-methyl-3-nitro-*muco*-inositol (50).[6c] Participation of the dialdehyde intermediate in the reaction pathway has been thus demonstrated. By use of this type of reactions, conversion of uronic acid derivatives leading to other type of cyclitols are currently under study in our laboratory.

4. Acetic Anhydride-Pyridine Degradation[7]

During the course of the structural study of sakuraso-saponin (1),[9] it was noticed that acetylation of 1 with acetic anhydride and pyridine (1:1) mixture under reflux for 1 hr did not give fully acetylated saponin but unexpectedly furnished 3,16-di-O-acetyl-protoprimulagenin A (51, 60%), 1,2,3,4,6-

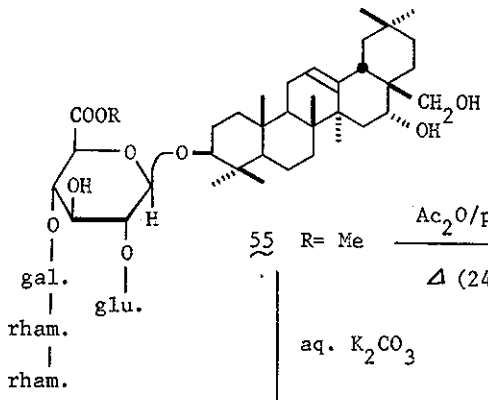

R
OMe
MeO
H
AcO
OH
37 R= β-OAc
38 R= α-OAc
42
CH_3NO_2/NaOMe
NO_2
48
49
50
sakuraso-saponin (1)
Ac_2O/py./Δ (1 hr)
Ac_2O/AcOH/BF_3/Δ (1 hr)
CH_2OAc
OAc
Me
51
52 R= β-OAc + α-OAc
53 R= β-OAc + α-OAc
54

penta-O-acetyl-glucopyranose (52, 78%), and trisaccharide nonaacetate (53, 71%). Thus, it has been noticed that heating with acetic anhydride-pyridine mixture can be a new degradation method for glucuronide-saponin.

To compare the method with ordinary acetolysis,[4b,23] sakuraso-saponin (1) has been treated with boron trifluoride etherate-acetic anhydride-acetic acid.[24] In this case, 1 yields 3,16,28-tri-O-acetyl-primulagenin A (54, 63%) in addition to 52 (71%) and 53 (65%), but no formation of 51 is observed. Therefore, the acetic anhydride-pyridine method is superior to ordinary acetolysis in regard to liberation of the acetylated genuine sapogenol (*e.g.* 51) from glucuronide-saponin (*e.g.* 1).

In order to elucidate the reaction pathway, the role of both reagents has been investigated. Treatment of sakuraso-saponin (1) with refluxing acetic anhydride-pyridine mixture of various molar ratios (1000:1 ~ 1:1000) has shown that mixtures of ratios (10:1 ~ 1:10) may effect the desired degradation (regardless of the yields) and both reagents are indispensable. For example, treatment of 1 with pyridine only unaffects saponin while treatment with boiling acetic anhydride results in formation of acetylated saponin.

Next, the role of free carboxyl group in the glucuronide moiety has been investigated. A methyl ester (55), which is prepared by methanolic hydrogen chloride treatment of 1, has been subjected to the acetic anhydride-pyridine degradation. Even after refluxing for 24 hr, no degradation product is formed but only a fully acetylated derivative (56) is obtained. On the other hand, when the acetic anhydride-pyridine degradation is applied to 57, in which the free carboxyl function is resumed, three degradation products (52, 53, 54) are obtained in high yields after refluxing for only 1 hr. Therefore, it has become clear that the degradation method is effected for cleavage at the glucuronide moiety having a free carboxyl function. In



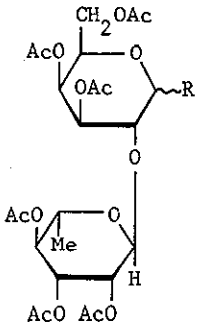
55 R= Me $\xrightarrow[\Delta\ (24\ hr)]{Ac_2O/py.}$ peracetate (56)

$\downarrow$ aq. K_2CO_3

57 R= H $\xrightarrow[\Delta\ (1\ hr)]{Ac_2O/py.}$ 52 + 53 + 54

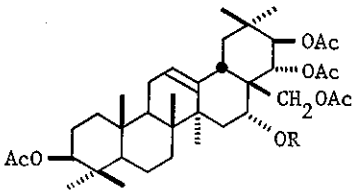
desacyl-jegosaponin (28)

$\xrightarrow{Ac_2O/py./\Delta\ (1\ hr)}$

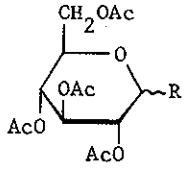


60 R= β-OAc

61 R= α-OAc



58 R= H, 59 R= Ac



52 R= β-OAc + α-OAc

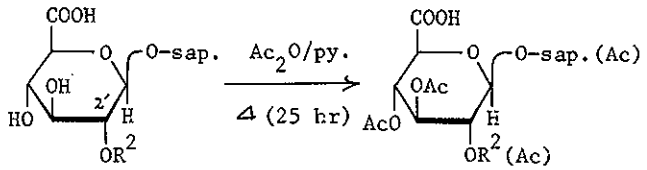
addition, since the methyl ester (55) is unaffected, the initial stage of the degradation seems to be started more likely with the decarboxylation reaction[25] than the β-elimination reaction.[4a]

The structure requirement in the glucuronide moiety has been next investigated. Desacyl-jegosaponin (28), which possesses two carbohydrate residues at C-2' and C-4' of the glucuronide moiety similarly as in sakurasosaponin (1) and its derivative (57), furnishes five degradation products after refluxing with acetic anhydride and pyridine for 1 hr: 3,21,22,28-tetra-O-acetyl-barringtogenol C (58, 49%) and 3,16,21,22,28-penta-O-acetyl-barringtogenol C (59, 12%) from the sapogenol portion and 52 (60%) and two disaccharide peracetates (60, 28% and 61, 35%) from the carbohydrate portion.

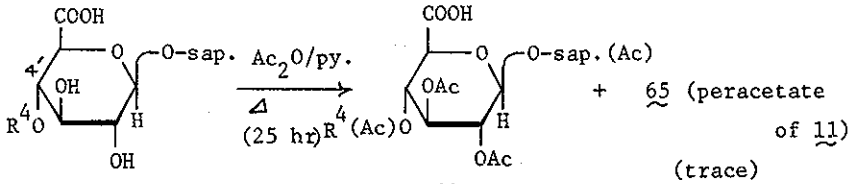
In the case of soyasaponin I (5) and chikusetsusaponin V (10), both of which possess only one carbohydrate residue at C-2' of the glucuronide moiety, no degradation product is obtained even after prolonged heating (refluxing for 25 hr with acetic anhydride and pyridine). The products are their peracetates (62, 63). On the other hand, chikusetsusaponin IV (9), which possesses a carbohydrate residue at C-4' of the glucuronide moiety, furnishes the peracetate (64) as the major product along with a trace amount of degradation product (65 = peracetate of 11).

Finally, the acetate mixture of sakuraso-saponin (1) (tridecaacetate and tetradecaacetate), which is prepared by treatment with acetic anhydride and pyridine at room temperature overnight, has been subjected to the degradation. The products are identical with those (51, 52, 53) obtained by direct degradation of sakuraso-saponin (1).

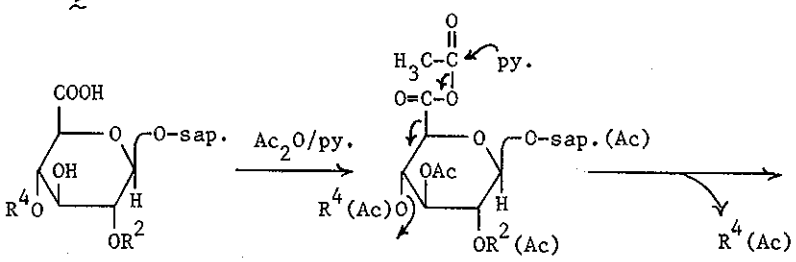
Based on these findings, the acetic anhydride-pyridine degradation method has been elucidated to be effective for degradation of glucuronide-saponin which contains, in the glucuronide moiety, a free carboxyl group and two



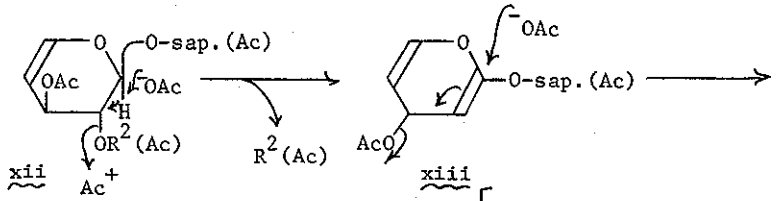
5, 10 62, 63



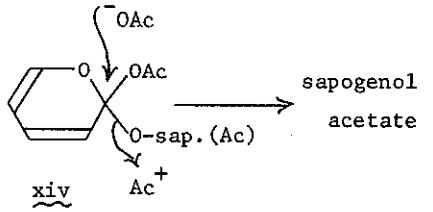
9 64



1, 28, 57 xi



xii xiii



xiv

sap.= sapogenol

sap.(Ac)= acetylated sapogenol

R^2, R^4= carbohydrate residue

$R^2(Ac)$, $R^4(Ac)$= acetylated carbohydrate residue

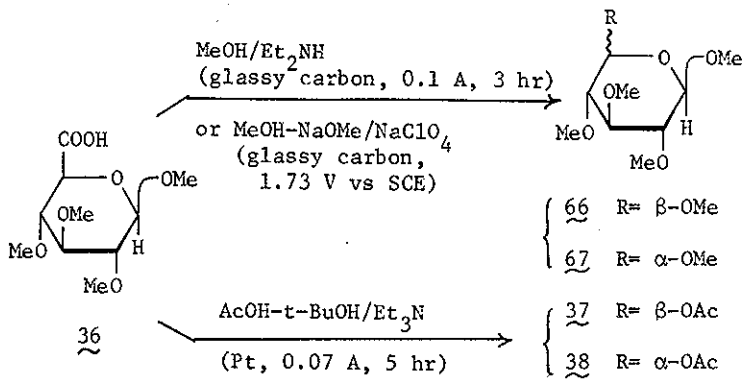
Scheme III

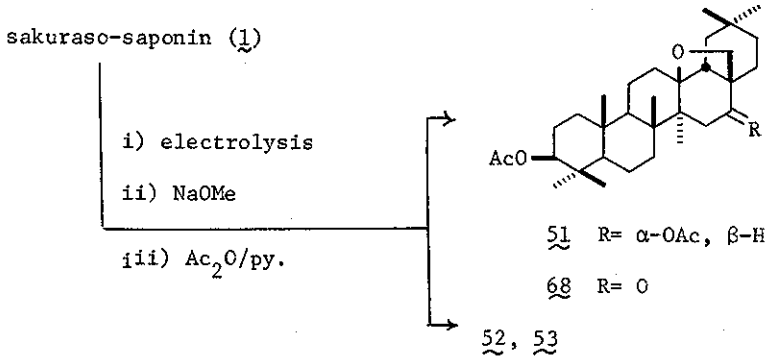
carbohydrate residues at C-2' and C-4'. The participation of the carbohydrate residue at C-3' is a subject of further study and any product derived from the glucuronide moiety has not yet been isolated. The reaction pathway is assumed to proceed as shown in scheme III, in which the reaction is initiated with mixed anhydride formation followed by decarboxylative elimination.

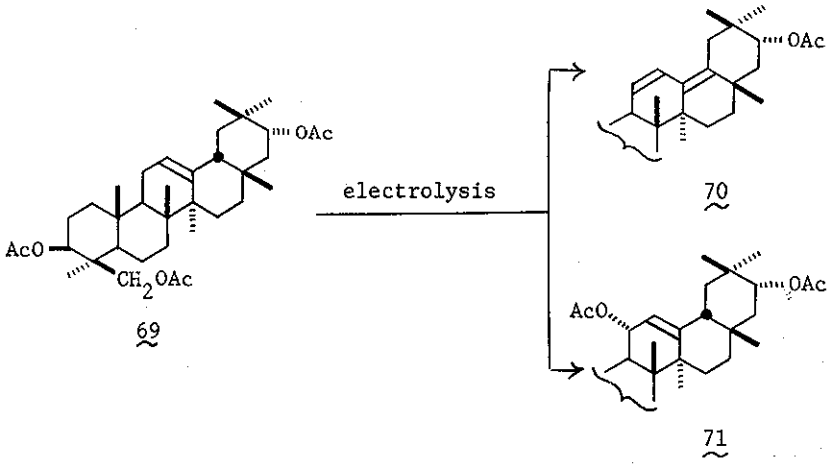
5. Anodic Oxidation[8]

In the lead tetraacetate degradation method described above, decarboxylation followed by introduction of an acetoxyl group at C-5' of the glucuronide moiety results in cleavage of the glucuronide linkage under alkaline conditions. Recently, it has been found that anodic oxidation is also a facile procedure for substitution of the carboxyl group in the glucuronide-saponin with some other residues (acetoxyl, methoxyl). The resulting acetoxylated or methoxylated derivative of glucuronide-saponin is readily decomposed to liberate the sapogenol. In regard to the anodic decarboxylation reaction, many works have been reported.[26] However, no report has been provided on anodic oxidation of the uronic acid derivative. Since the work in our laboratory is currently in progress, some examples are given below.

When methyl 2,3,4-tri-O-methyl-β-D-glucopyranosiduronic acid (36) in methanol-diethylamine is subjected to constant current electrolysis (0.1 A, 15-25 V) using glassy carbon as an anode, two methoxylated derivatives (66, 37% and 67, 42%) are obtained. The same products (66, 30% and 67, 34%) are also obtained, when the reaction is undertaken by controlled potential electrolysis (1.73 V vs S.C.E.) in methanol-sodium methoxide mixture using sodium perchlorate as the supporting electrolyte.


MeOH/Et2NH
(glassy carbon, 0.1 A, 3 hr)
or MeOH-NaOMe/NaClO4
(glassy carbon,
1.73 V vs SCE)
COOH
OMe
MeO
H
MeO
36
R
OMe
MeO
H
MeO
66 R= β-OMe
67 R= α-OMe
AcOH-t-BuOH/Et3N
(Pt, 0.07 A, 5 hr)
37 R= β-OAc
38 R= α-OAc

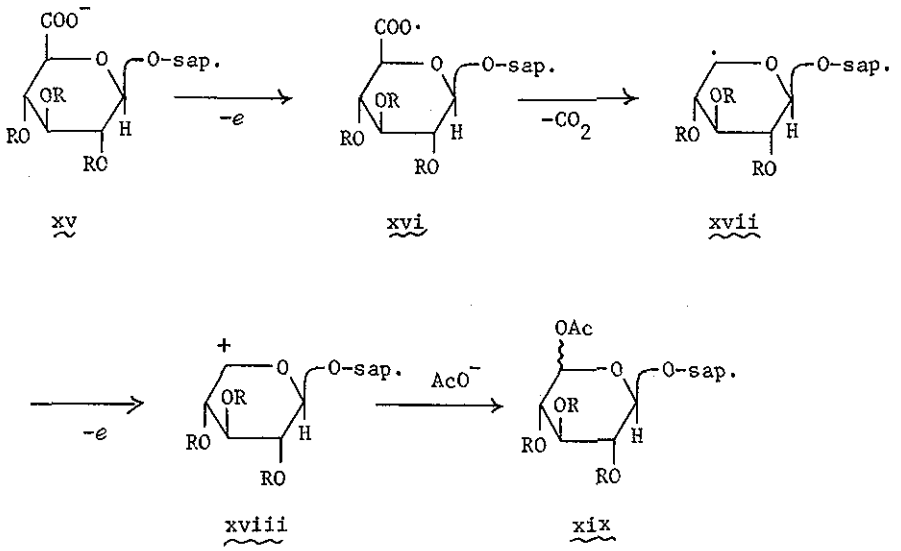

sakuraso-saponin (1)
i) electrolysis
ii) NaOMe
iii) Ac2O/py.
AcO
O
R
51 R= α-OAc, β-H
68 R= O
52, 53


OAc
AcO
CH2OAc
69
electrolysis
OAc
70
AcO
OAc
71

On the other hand, constant current electrolysis (0.07 A, 20-25 V) of 36 with a platinum electrode in acetic acid-t-butanol-diethylamine furnishes two acetoxylated derivatives (37, 34% and 38, 35%), thus anodic oxidation has been found to be useful for decarboxylation followed by introduction of an acetoxyl or a methoxyl group at the glucuronide moiety, which is a promising process for cleavage of the glucuronide linkage.

When sakuraso-saponin (1) is subjected to constant current electrolysis (0.14 A, 45-58 V) in acetic acid-triethylamine, an acetoxylated product is formed. Alkaline treatment (sodium methoxide-methanol) followed by acetylation (acetic anhydride-pyridine) of the product gives four degradation products: 3,16-di-O-acetyl-protoprimulagenin A (51, 21%) and 3-O-acetyl-aegicerin (68 = acetate of 4, 27%) from the sapogenol portion and 52 (48%) and 53 (36%) from the carbohydrate portion. Although yields of the products are yet unsatisfactory, it has been shown that anodic oxidation is applicable for selective cleavage of the glucuronide linkage similarly as the lead tetraacetate degradation. However, as noticed in formation of an oxidized product (68), anodic oxidation may accompany concomitant oxidation of the hydroxyl group (especially axial one) in the sapogenol portion. In fact, anodic oxidation (constant current electrolysis) of protoprimulagenin A (2) under similar conditions furnishes aegicerin (4) in a 65% yield.

Next, the peracetate (62) of soyasaponin I (5) has been subjected to constant current electrolysis. In this case, although two disaccharide peracetates (60, 26% and 61, 23%) are liberated from the carbohydrate portion as expected, the sapogenol portion yields an inseparable mixture due to concomitant side reactions. To clarify the reason, 3,21,24-tri-O-acetyl-soyasapogenol B (69) has been subjected to anodic oxidation under the same reaction conditions. After 1 hr, two products (70, 5% and 71, 75%) are



i) NaOMe
⟶ products (sapogenol acetate, acetylated carbohydrate)
ii) Ac_2O/py.

Scheme IV R= H, Ac, or carbohydrate residue

yielded.

It has been noticed so far that anodic oxidation may be suitable for liberation of the carbohydrate residues which attach to the glucuronide moiety of glucuronide-saponin. The reaction pathway is presumed to proceed through radical (xvi, xvii) and cation (xviii) which are derived *via* one-electron or two-electron oxidation as shown in scheme IV and is analogous to ordinary electrolytic decarboxylation.[27]

6. Conclusion

Based on considerations of the reaction pathway, above-described four degradation methods, which effect selective cleavage of the glucuronide linkage in glucuronide-saponin, seem to be applicable also for the cleavage of other kinds of uronide linkages. Some of the reaction intermediates derived from the glucuronic acid moiety are presumed to be potential species for conversion of glucuronic acid to some other type of carbohydrate derivatives. Studies on these subjects are now in progress in our laboratory.

Among four methods, the lead tetraacetate degradation liberates all the constituents of the starting glucuronide-saponin in high yields except the diene from the glucuronic acid moiety. It may be preferable for selective degradation at the glucuronide moiety in polysaccharides and will be useful for structural studies.

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