Journal of Chromatography, 118 (1976) 201–216

CHROM. 8796

STUDIES ON MONOTERPENE GLUCOSIDES AND RELATED NATURAL PRODUCTS

XXXI*. GAS CHROMATOGRAPHY AND GAS CHROMATOGRAPHY–MASS SPECTROMETRY OF IRIDOID AND SECOIRIDOID GLUCOSIDES

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(First received August 5th, 1975; revised manuscript received October 7th, 1975)

SUMMARY

A total of 33 iridoid and secoiridoid glucosides were detected by gas chromatography on several columns such as OV-1 or OV-17. Representative glucosides were then subjected to gas chromatography–mass spectrometry, giving some characteristic peaks that permitted the discrimination of both types of glucosides from other compounds in most instances. The successful detection of both types of glucosides in several plant extracts showed the applicability of this combination of methods to small amounts of plant materials.

INTRODUCTION

On account of the rapid increase in the number of newly found iridoid and secoiridoid glucosides in recent years, the total number of these glucosides is now estimated to be more than one hundred[2–4]. The outline of the biosynthetic pathway of these glucosides has also been clarified recently[5]. On the other hand, these glucosides and related compounds, such as indole alkaloids, have been found to be distributed widely in dicotyledons, especially in sympetalous plants. Accordingly, chemotaxonomic studies on iridoids have also been carried out in recent years[6–10]. However, in performing such work, special attention has to be paid to the accurate detection of the substances involved and also an exhaustive examination of as many plants as possible has to be made. The rapid examination of iridoid glucosides in many plant sources has so far been dependent almost entirely upon thin-layer and paper chromatography. However, in our experience, these methods alone have been of no practical value in

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detecting substances in small amounts of plant materials, except for highly unsaturated glucosides such as asperuloside (18)*, which develops a blue colour on the addition of mineral acids. We therefore studied the application of gas chromatography (GC) in this field. Although the GC examination of iridoids such as aucubin (1), monotropein (5), catalposide (20) and several other glycosides has been reported[11], no systematic work on many glucosides of the iridoid and secoiridoid type has, to our knowledge, been reported.

We examined 33 iridoid and secoiridoid glucosides of known structures as their trimethylsilyl derivatives and succeeded in detecting all of them. The examination by gas chromatography–mass spectrometry (GC–MS) of several representative glucosides was then carried out in order to establish a means of confirming whether or not a particular peak in a chromatogram is truly ascribable to an iridoid or a secoiridoid glucoside, and to prepare for the detection of new glucosides. We found that most glucosides can be distinguished by this method with the aid of the characteristic fragmentation patterns.

After confirming the applicability of the GC method to some plants containing known glucosides, we examined plants whose constituents were unknown by GC and GC–MS, and succeeded in detecting iridoid and secoiridoid glucosides including a few new substances.

EXPERIMENTAL

Apparatus

Gas chromatography. A Shimadzu Model GC-1C gas chromatograph with a hydrogen FID-1B flame ionization detector was used under the following conditions: carrier gas nitrogen at a flow rate of 60 ml/min; inlet pressure, 1.2–2.0 kg/cm^2; hydrogen flow-rate, 35–40 ml/min; sensitivity, $10^3\,\Omega$; range, 0.8–6.4 V; detector temperature, 290° (experiments in Table I) and 310° (experiments in Table II); injection port temperature, 300°; U-shaped glass column, 1.8 m × 4 mm I.D. or 0.5 m × 3 mm I.D.; packing, 1.5% OV-1, 1.5% OV-17, 2% OV-210 and 2% OV-225 on 80–100-mesh Shimalite W AW/DMCS.

Before use, the column was detached from the detector and baked under a stream of nitrogen (flow-rate 20 ml/min) at 270–300° for 24 h in order to stabilize the baseline.

Gas chromatography–mass spectrometry. A Hitachi K-53 gas chromatograph and a Hitachi RMU-6 E mass spectrometer were used. The following conditions were applied: column temperature, 240–260°; carrier gas, helium at a flow-rate of 130 ml/min; glass column, 0.5 m × 3 mm I.D.; packing, 1.5% OV-17 on 80–100-mesh Shimalite W AW/DMCS for common glucosides and plant extracts and 1.5% OV-1 on 80–100-mesh Shimalite W AW/DMCS for oleuropein-type glucosides; ion source temperature, 200–260°; ionizing voltage, 70 or 10–20 eV; total emission, 80 μA; target current, 70 μA; accelerating voltage, 1800 V; multiplier potential, 1.5–3.0 kV; scanning speed, 4 sec (*m/e* 600).

* The numbers in parentheses throughout the text and in Tables I and II, and the numbered peaks in Figs. 11–16, refer to the numbers assigned to the structural formulae of the glucosides examined, as shown in Figs. 1 and 2.

Materials

The iridoid glucosides and secoiridoid glucosides studied are listed in Tables I and II and their structures are shown in Figs. 1 and 2. Most of the glucosides were isolated during the course of this series of studies carried out at the Department of Phytochemistry, Faculty of Pharmaceutical Sciences, Kyoto University.

Preparation of samples from plant materials

A plant material (3–5 g) was cut into pieces and extracted with two 50-ml portions of hot water for 20 min. The combined extracts were poured into a column of charcoal (active carbon for column chromatography, Wako Pure Chemicals, Osaka, Japan) consisting of half the amount of the fresh plant material, and the sugars were removed by elution with 500 ml of water. The column was then eluted with 200 ml of methanol and the eluate was concentrated *in vacuo*. The residue was dried at 50° for at least 2 h under reduced pressure giving a foamy powder (A), which was used as a sample.

Preparation of trimethylsilyl derivatives

A mixture of anhydrous pyridine (10 ml), hexamethyldisilazane (HMDS, Tokyo Kasei Kogyo Co., Tokyo, Japan; 2 ml) and trimethylchlorosilane (TMCS, Tokyo Kasei Kogyo Co., Tokyo, Japan; 1 ml) was used for trimethylsilylation. A sample of a glucoside (or glucoside mixture) (0.5 mg) mixed with the reagent solution (50 μl) was heated in a stoppered glass tube at 80° for over 5 min and a 0.2–2-μl aliquot of the solution was injected into the gas chromatograph.

For trimethylsilylation of the plant extract, the foamy powder A described above (3 mg) was mixed with the reagent solution (300 μl) and heated at *ca.* 80° for more than 5 min. The reaction mixture was concentrated to dryness *in vacuo* at 70°, giving a syrupy residue that was dissolved in chloroform (30 μl). A 2–4-μl aliquot of the chloroform solution was injected into the gas chromatograph.

RESULTS AND DISCUSSION

Gas chromatography of trimethylsilyl (TMS) ethers of glucosides

The results of the chromatography on the columns of OV-17, OV-1, OV-210 or OV-225 operated at a column temperature of 270°, 230° or 215° are shown in Table I. All of the TMS-glucosides tested showed longer retention times than that of sucrose. On the other hand, as aucubin (1) showed the shortest retention time of these glucosides on each column, the retention time of TMS-aucubin was used as the basis for relative retention times in each instance.

Although most glucosides were detected on a 1.5% OV-17 or 1.5% OV-1 column, each 1.8 m long, at 270°, paederoside (19), catalposide (20), oleuropein (31), etc., were not detected. Amaroswerin (28) and amarogentin (29) were detected on the OV-17 column, but were undetectable on the OV-1 column. In general, the slightly polar OV-17 column gave larger differences in retention times among various compounds than did the non-polar OV-1 column; the range of the retention values for the glucosides tested were 1.00–3.08 on OV-1 and 1.00–6.70 on OV-17. However, the OV-1 column gave some better results, depending on the combination of glucosides, such as the pair loganin (7) and secologanin (21).

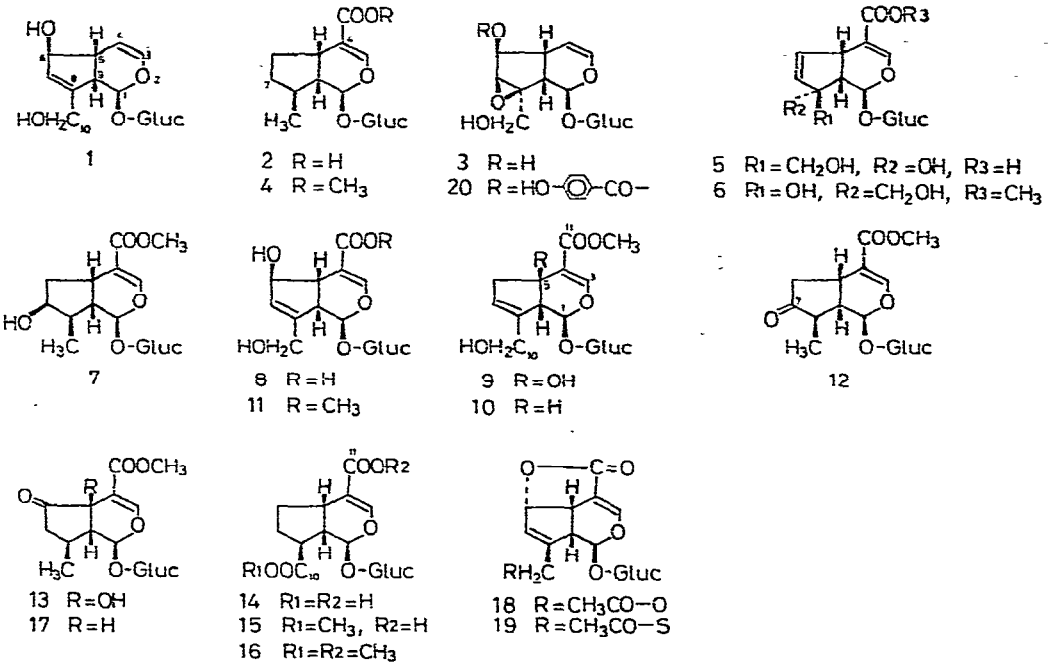

Fig. 1. Structures of the iridoid glucosides examined.

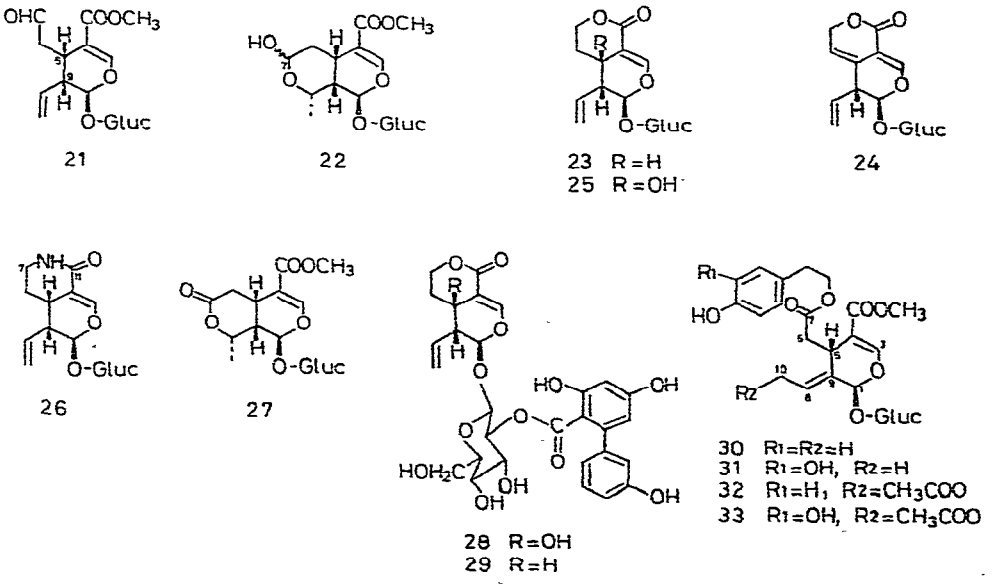

Fig. 2. Structures of the secoiridoid glucosides examined.

TABLE I

RELATIVE RETENTION TIMES OF TMS-GLUCOSIDES

*Glucoside**	*1.5% OV-17 column, 1.8 m, 270°*	*1.5% OV-1 column, 1.8 m, 270°*	*1.5% OV-17 column, 0.5 m, 230°*	*2% OV-210 column, 0.5 m, 215°*	*2% OV-225 column, 0.5 m, 230°*
Sucrose	0.48	0.79	0.41	0.54	0.44
Aucubin (1)	1.00 (7.12 min)	1.00 (7.65 min)	1.00 (3.50 min)	1.00 (1.32 min)	1.00 (2.70 min)
7-Deoxyloganic acid (2)	1.37	1.18	1.40	1.88	1.65
Catalpol (3)	1.37	1.28	1.40	2.02	1.75
7-Deoxyloganin (4)	1.39	1.02	1.35	1.97	2.07
Monotropein (5)	1.57	1.65	1.73	2.33	1.89
Gardenoside (6)	1.65	1.52	1.81	2.41	1.95
Secologanin (21)	1.75	1.16	1.85	3.67	2.45
Loganin (7)	1.78	1.52	1.92	2.56	2.25
Scandoside (8)	1.98	2.00	2.31	2.43	2.51
Theviridoside (9)	2.02	1.78	2.32	2.88	2.55
Geniposide (10)	2.15	1.65	2.42	3.17	2.89
Scandoside methyl ester (11)	2.19	1.83	2.59	3.08	2.60
7-Dehydrologanin (12)	2.30	1.39	2.60	6.32	5.35
Morroniside (22)	2.53	1.96	2.91	3.63	3.37
Hastatoside (13)	2.55	1.70	2.90	5.19	4.46
Forsythide (14)	2.55	2.30	3.00	4.68	4.24
Forsythide 10-methyl ester (15)	2.74	2.12	3.20	4.93	4.52
Forsythide dimethyl ester (16)	2.75	1.75	3.20	5.07	4.56
Verbenalin (17)	2.75	1.60	3.20	7.44	6.35
Sweroside (23)	3.07	1.60	3.21	11.88	8.75
Gentiopicroside (24)	3.08	1.63	3.22	9.56	8.25
Swertiamarin (25)	3.08	1.57	3.31	9.00	7.08
Bakankosin (26)	5.31	2.16	5.86	13.55	14.75
Kingiside (27)	5.34	2.61	7.10	20.06	18.59
Amaroswerin (28)	6.18	—	8.20	—	—
Amarogentin (29)	6.21	—	8.30	—	—
Asperuloside (18)	6.70	3.08	9.43	20.06	16.06
Paederoside (19)	—	—	—	—	—
Ligustroside (30)	—	—	—	—	—
Catalposide (20)	—	—	—	—	—
Oleuropein (31)	—	—	—	—	—
10-Acetoxyligustroside (32)	—	—	—	—	—
10-Acetoxyoleuropein (33)	—	—	—	—	—

* The numbers in parentheses refer to the numbered structures listed in Figs. 1 and 2.

On the basis of the above results, we carried out experiments employing a 1.5% OV-17 column, 0.5 m long, at 230°. In this instance, the reduction in retention time resulted in the sharpening of each peak. However, pairs such as gardenoside (6) and loganin (7), theviridoside (9) and geniposide (10), forsythide (14) and forsythide 10-methyl ester (15), etc., were less well resolved on this column than on the longer one because of the contiguous retention times.

Next, analyses were carried out with more polar liquid phases, consisting of 0.5-m columns of OV-210 at 215° and OV-225 at 230°. Of particular significance in

these instances were the longer retention times of keto compounds such as 7-dehydrologanin (12) and verbenalin (17) and also lactonic compounds such as sweroside (23), gentiopicroside (24) and kingiside (27), compared with those observed on the OV-17 column. A clear separation of sweroside (23) and gentiopicroside (24) was observed on OV-210, while they were insufficiently resolved on the other columns.

Under the conditions so far described, glucosides such as paederoside (19) and oleuropein (31) were not detected, and amaroswerin (28) and amarogentin (29) were also difficult to detect on columns other than OV-17. The GC of these samples was therefore attempted on shorter OV-17 and OV-1 columns (0.5 m) at 270°. As shown in Table II, these glucosides were well separated under these conditions, which are, however, unsuitable for other glucosides such as a mixture consisting of scandoside (8), asperuloside (18) and paederoside (19), which will be considered later, because the retention time of scandoside (8) was too short. In such a case, however, satisfactory results were obtained by the application of programmed-temperature GC. Overall, the GC detection of all of the glucosides examined was successful under suitably selected conditions.

TABLE II

RETENTION TIMES (min) OF SPARINGLY VOLATILE TMS-GLUCOSIDES

*Glucoside**	*1.5% OV-17 column, 0.5 m, 270°*	*1.5% OV-1 column, 0.5 m, 270°*
Amaroswerin (28)	4.40	2.80
Amarogentin (29)	4.40	3.00
Asperuloside (18)	5.12	2.40
Paederoside (19)	10.80	4.20
Ligustroside (30)	22.00	10.00
Catalposide (20)	24.32	12.20
Oleuropein (31)	28.40	13.20
10-Acetoxyligustroside (32)	42.00	15.80
10-Acetoxyoleuropein (33)	56.80	22.00

* The numbers in parentheses refer to the numbered structures listed in Figs. 1 and 2.

Gas chromatography–mass spectrometry of TMS ethers of glucosides

Although several papers on the mass spectrometry of iridoid glucoside have been published[12–16], no systematic study of a series of these substances has been carried out except that reported by Bentley *et al.*[17]. From the previous findings, however, it is found that molecular ion peaks of glucosides, if any, are very weak and the main peaks are assignable to aglucone or sugar moieties. In particular, Bentley *et al.*[17] reported that the mass spectra (70 eV) of glucosides indicated, in addition to ion A due to the aglucone, ion B derived solely from the dihydropyran portion (Fig. 3).

TMS derivatives of the following three groups of iridoid glucosides were further subjected to GC–MS at 70 eV: the first group of compounds with a carbomethoxy group at the C-4 position, including 7-deoxyloganin (4), gardenoside (6), loganin (7), theviridoside (9), geniposide (10), scandoside methyl ester (11), forsythide dimethyl ester (16) and verbenalin (17); the second group of compounds without a substituent

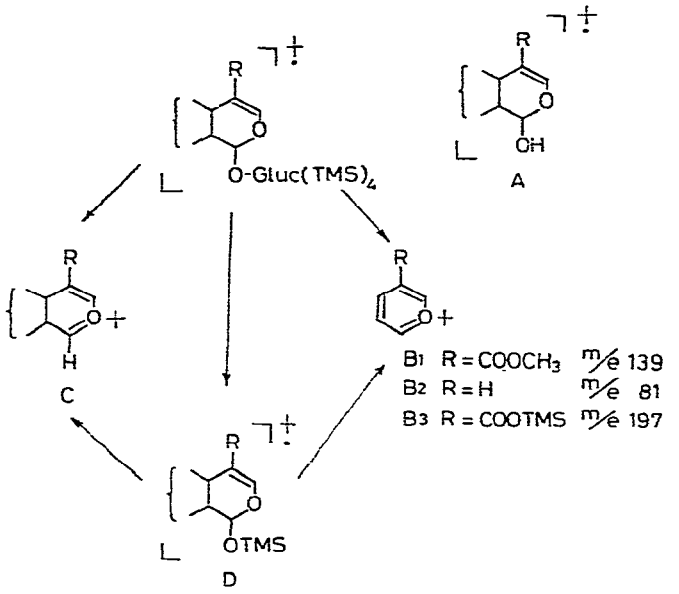

Fig. 3. Fragmentation pattern of iridoid and secoiridoid glucosides.

at the C-4 position, including aucubin (1) and catalpol (3); and the third group of compounds having a carboxyl group at the C-4 position, such as 7-deoxyloganic acid (2), monotropein (5) and forsythide 10-methyl ester (15). In this instance, contrary to the MS of the free glucosides, ion A due to the aglucone was not observed, while ion B resulting from the dihydropyran portion of the aglucone and ion C formed by the elimination of the sugar moiety from the glucoside were observed.

Because of the retention of the aglucone ring structure of the original glucosides, the mass number of ion C is characteristic of each glucoside. On the contrary, as already reported[17], ion B, arising solely from the dihydropyran ring portion, gave a peak at *m/e* 139 (peak B_1), 81 (peak B_2) or 197 (peak B_3) when R was $COOCH_3$, H or COOH, respectively (COOTMS in the TMS derivative). However, glucosides with a hydroxyl group at the C-5 position, such as theviridoside (9) and hastatoside (13), gave a very weak peak of ion B_1, although that of ion C was clearly observed. This result is due to the different mode of cleavage at the ring junction caused by the presence of the hydroxyl group.

With all of the glucosides, several peaks due to the sugar moiety were detected at *m/e* 361, 271, 243, 217, 204, 191, 169, 147, 129, 103, 73, etc. Most of these peaks were also observed in the MS of the TMS ether of glucose[18], while the intensities were considerably different from those of the glucosides. The peak at *m/e* 361 was particularly distinguishable as the base peak of many glucosides*.

The mass spectra of these substances were then measured at 17 eV, giving much simpler peak patterns than those observed at 70 eV for the glucosides themselves[17]. These TMS-glucosides gave predominantly ion D, which could be formed by

* With oleuropein-type glucosides, another ion gave the base peak, which is described later.

the loss of a sugar moiety followed by the rearrangement of TMS together with ion C, which could be formed either from D through the elimination of a TMSO group or directly from the TMS-glucosides by the loss of the sugar portion. Although peak D of compounds such as 7-deoxyloganic acid (2) and theviridoside (9) was detected even at 70 eV, the intensity was far weaker than that at 17 eV. Monotropein (5) gave peak D–15 (CH_3) instead of peak D, while peak C was observed as usual. The predominance of ions C and D at low energies indicates the facile formation and also the instability of these ions. At such low energies, peak B was not observed and the relative intensities of the peaks that originated from the sugar moiety to the base peak at *m/e* 361 decreased remarkably. Thus the fragments 451, 361, 331, 319, 271, 243, 217, 204, 191 and 169 were observed whereas smaller fragments were undetectable. In order to locate the TMS group in the sugar moiety which transferred to the C-1 position of D, the TMS derivatives of the 2′-methyl, 3′-methyl and 2′,3′-dimethyl ethers[19,20] of 7-deoxyloganin (4) were subjected to GC–MS, peak D being found in each instance. Accordingly, it seems likely that the TMS group could have been transferred not exclusively from the adjacent C–2′ or C–3′ position. The possibility that ion D is formed by the rearrangement of TMS in the atmosphere of the column during the GC–MS measurement or that the ion is a decomposition product of the samples during GC was excluded by the appearance of peak D in the mass spectra of some TMS-glucosides taken with a direct inlet system under the following conditions: (a) Hitachi RMU-6M mass spectrometer, evaporation temperature 100°, ionizing voltage 15 eV, accelerating voltage 3.2 kV (maximum *m/e* 1500), ion source temperature 150°; (b) Jeol JMS-OISG mass spectrometer, evaporation temperature 100°, ionizing voltage 10 eV, accelerating voltage 3.0 kV, ion source temperature 100°. To our knowledge, it has not been reported that the peak of the aglucone-TMS ion resulting from the transfer of TMS from the sugar to the aglucone moiety appeared in the mass spectra of any TMS-glucoside. We recognized, however, the prominent appearance of this peak also with TMS derivatives of hydroquinone-type glucosides such as pirolatin, homoarbutin and methylarbutin even at 70 eV.

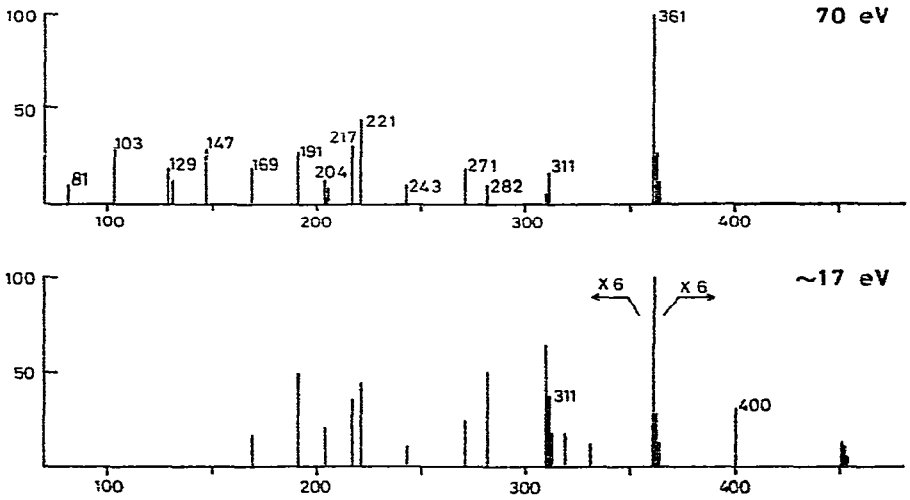

Fig. 4. Mass spectra of TMS ether of aucubin (1) at 70 and *ca.* 17 eV obtained by GC–MS.

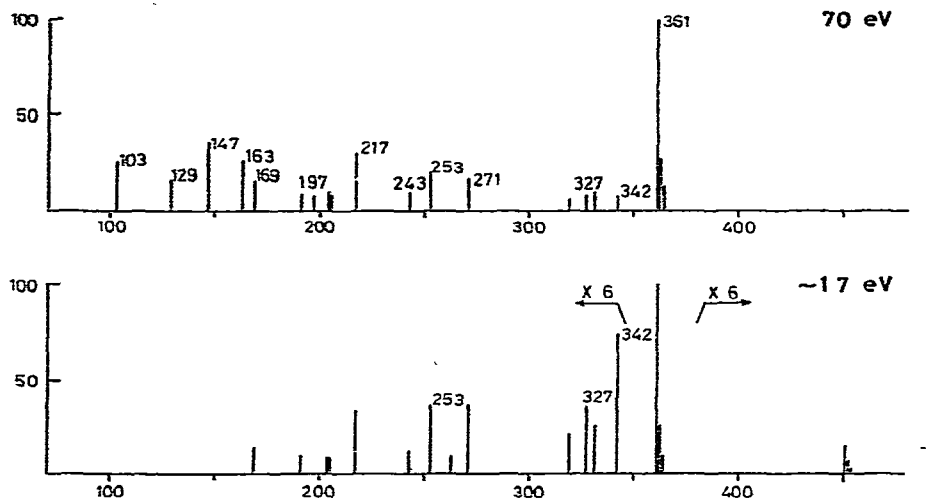

Fig. 5. Mass spectra of TMS derivative of 7-deoxyloganic acid (2) at 70 and *ca.* 17 eV obtained by GC–MS.

The mass spectra of aucubin (1), 7-deoxyloganic acid (2) and loganin (7), measured at 70 and 17 eV, are shown in Figs. 4–6.

Next, GC–MS was carried out on the secoiridoid glucosides sweroside (23), gentiopicroside (24) (Fig. 7) and bakankosin (26), with a lactone or lactam ring between positions 7 and 11, morroniside (22) (Fig. 8), which contains a hemiacetal ring between positions 7 and 8, and secologanin (21), which lacks any bonding between positions 7 and 11 and positions 7 and 8. All of the spectra of these glucosides run at 70 eV showed the significant common peaks corresponding to ion C described above, together with peaks that originated from the sugar moiety. In the sweroside group,

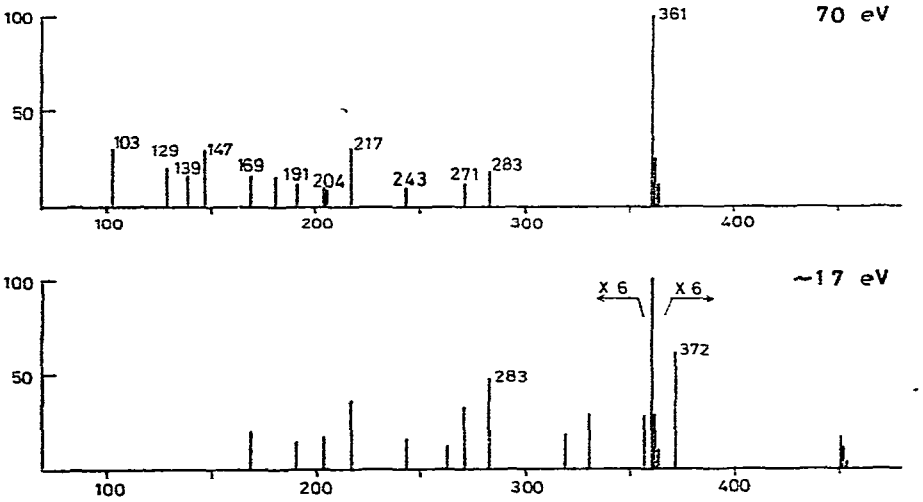

Fig. 6. Mass spectra of TMS ether of loganin (7) at 70 and *ca.* 17 eV obtained by GC–MS.

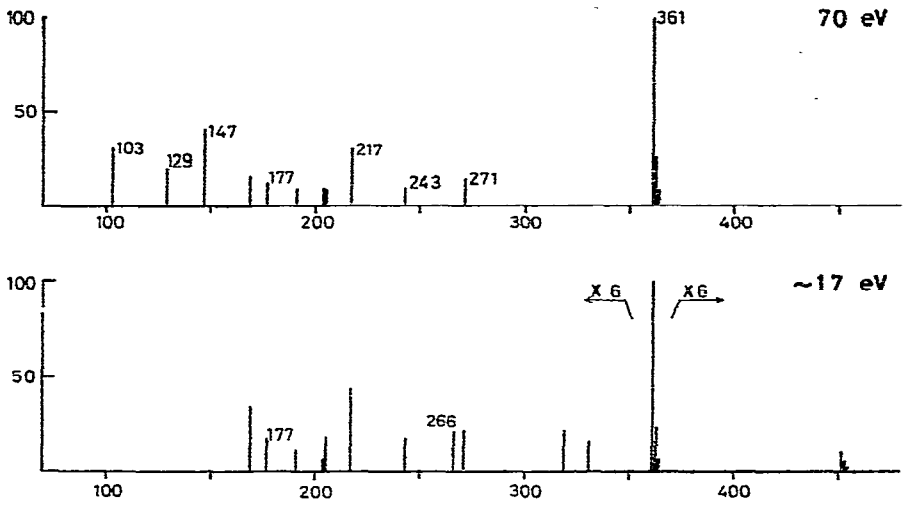

Fig. 7. Mass spectra of TMS ether of gentiopicroside (24) at 70 and *ca.* 17 eV obtained by GC–MS.

no significant peak resulting from the aglucone moiety was observed except peak C. With morroniside (22), peak B_1 was much more conspicuous than this peak. With secologanin (21), peak B_1 was weak, while a conspicuous peak of ion E (Fig. 9) formed by the McLafferty-type rearrangement was observed. At 17 eV, all of these secoiridoid glucosides also gave prominent peaks assignable to ions C and D. Secologanin (21) showed, in addition to these peaks, peak E of very high intensity.

GC–MS of oleuropein-type glucosides that possess an ethylidene or acetoxyethylidene side-chain, such as ligustroside (30), oleuropein (31) (Fig. 10), 10-acetoxy-

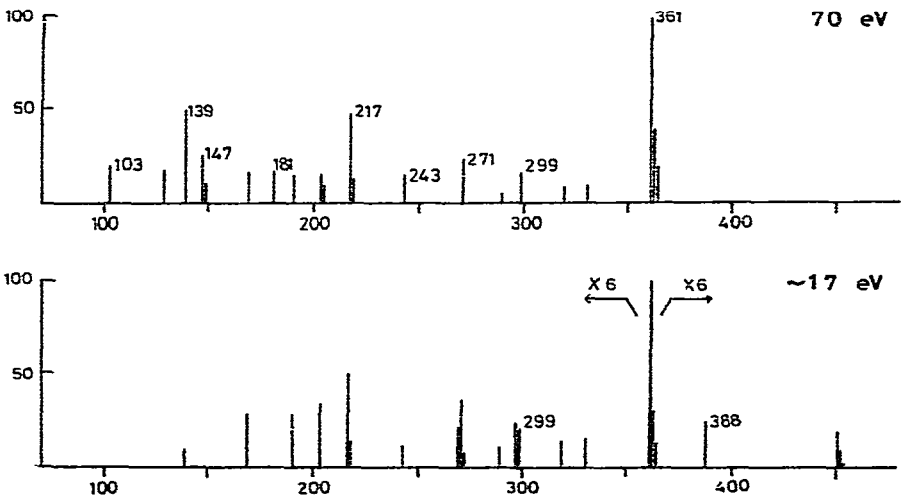

Fig. 8. Mass spectra of TMS ether of morroniside (22) at 70 and *ca.* 17 eV obtained by GC–MS. It is known that morroniside occurs as a mixture of both 7-epimers. The mass spectral patterns of the peaks of both epimers in the chromatogram are superimposable.

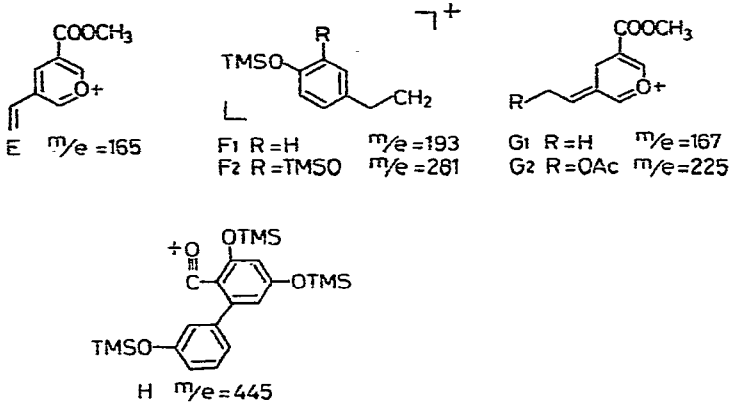

Fig. 9. Other fragment ions from secoiridoid glucosides.

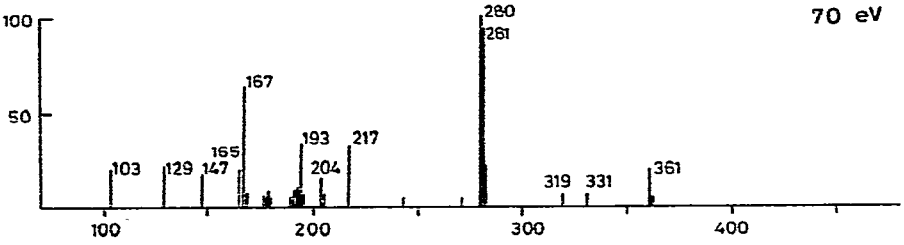

Fig. 10. Mass spectrum of TMS ether of oleuropein (31) at 70 eV obtained by GC–MS.

ligustroside (32) and 10-acetoxyoleuropein (33) were then measured at 70 eV. Contrary to the behaviour of other glucosides, the glucosides of this type showed characteristically the ion F_1, F_2 or that formed by the elimination of hydrogen from the latter ion as the base peak, each of them originating from the phenethyl alcohol portion, while several peaks due to the sugar moiety appeared with low intensities. Although the tropylium ion at *m/e* 179 or 267, corresponding to F_1 or F_2, is also observed, the intensity is much weaker in comparison with ordinary cases. Other significant peaks are that at *m/e* 167 due to ion G_1, which seems to have been formed by the elimination of the C-5 substituent and the subsequent rearrangement of a hydrogen in the case of compounds 30 and 31*, and that at *m/e* 165 due to ion E, which is considered to be formed by the elimination of the C-5 substituent followed by the migration of the double bond and the loss of a hydrogen or acetoxy group. In the 10-acetoxy-type glucosides, ion G_2, retaining the 10-acetoxy group, also appears as a peak with low intensity compared with that of G_1. Glucosides of this type do not give the peak corresponding to B_1.

The GC–MS examination of some substances was not satisfactory. In spite of the use of various conditions, the TMS ethers of asperuloside (18) and paederoside (19) did not give any significant peak other than those due to the sugar moiety. Ama-

* With compounds 32 and 33 the elimination of the acetoxy group at the C-10 position and the migration of a hydrogen atom to the C-10 position would occur together with these reactions.

roswerin (28) and amarogaentin (29) gave the same spectral pattern, showing only peak H due to the biphenylcarboxylic acid portion and peaks that originated from the sugar moiety.

The spectral patterns obtained by the direct measurement of the mass spectra of the TMS ethers of the pairs asperuloside (18) and paederoside (19), and amaroswerin (28) and amarogentin (29), were identical, although the TMS ethers of these compounds had different retention times. The separation of these substances by GC–MS was therefore unsuccessful, but it is most likely that each GC peak is not due to the degradation product because a definite single peak appeared in each chromatogram.

As discussed above, the application of GC–MS to the detection and separation of iridoid and secoiridoid glucosides has not yet covered all substances in both groups. However, on examining new glucosides, peaks B, C, D and E (especially B for the iridoid glucosides and B and E for the secoiridoid glucosides), together with several peaks originating from the sugar moiety, could serve as characteristics for distinguishing glucosides of these types from other substances. In particular, as several glucosides of these series often coexist in a single plant, the detection of even one species could give a clue to the discovery of new glucosides.

Investigation of glucosides in plant extracts by gas chromatography

Known glucoside-containing plant materials employed were the leaves of *Paederia scandens* (Lour.) Merill var. *mairei* (Léveillé) Hara (Rubiaceae) collected in Kyoto in the middle of September and the leaves of *Lonicera morrowii* A. Gray (Caprifoliaceae) collected in Kyoto at the end of June. The former contain scandoside (8), asperuloside (18) and paederoside (19)[21], while the latter contain secologanin (21) and sweroside (23)[22].

Leaves of the following plants which are related to iridoid-containing plants were then selected as examples of plants whose constituents were unknown: *Adina pilulifera* (Lam.) Franch. ex Drake (Rubiaceae) collected in Kagoshima at the end of October, *Adina racemosa* (Sieb. et Zucc.) Miq. collected in Okinawa at the end of June, *Allamanda cathartica* var. *schotti* (Pohl) Rafill (Apoxynaceae) cultivated in a

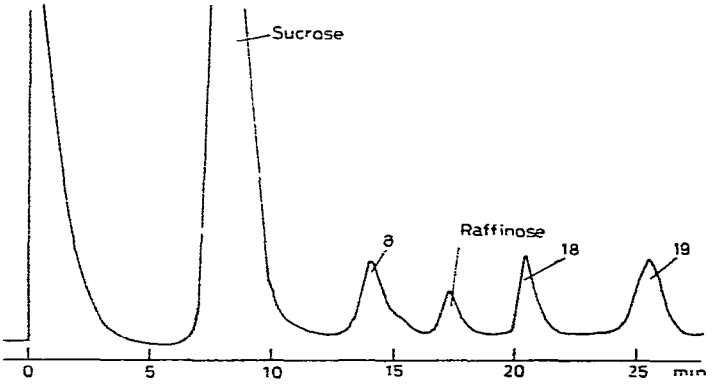

Fig. 11. Chromatogram of the extract of *Paederia scandens* on a 1.5% OV-17 column (0.5 m), temperature programmed from 200 to 280° at 4°/min and then maintained at 280°. The numbered peaks in Figs. 11–16 refer to the numbered structures listed in Figs. 1 and 2.

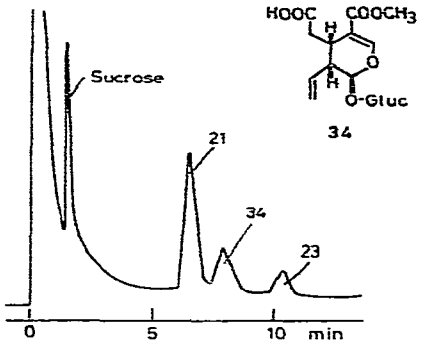

Fig. 12. Chromatogram of the extract of *Lonicera morrowii* on a 1.5% OV-17 column (0.5 m) at 230°.

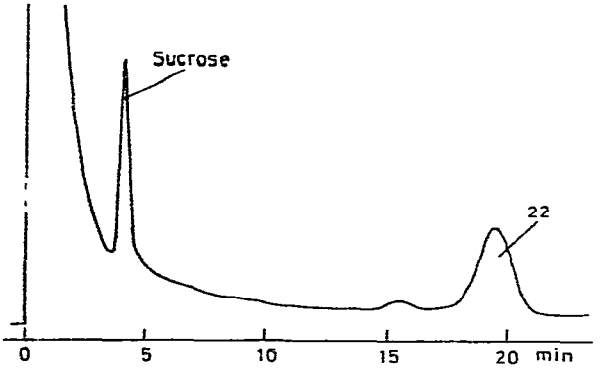

Fig. 13. Chromatogram of the extract of *Adina pilulifera* on a 1.5% OV-17 column (1.8 m) at 260°.

greenhouse and *Osmantus heterophyllus* (G. Don) P. S. Green (Oleaceae) collected in Kyoto in the middle of May.

Trimethylsilylated plant extracts prepared according to the method described under Experimental were subjected to GC in order to identify the constituents with the glucosides shown in Tables I and II. Unidentified substances were examined by GC–MS. For the reasons indicated earlier, both OV-17 and OV-1 columns were employed under various conditions.

GC of the extract of *P. scandens* showed peaks due to the glucosides scandoside (8), asperuloside (18) and paederoside (19), together with raffinose* (Fig. 11).

In *L. morrowii*, secologanin (21) and sweroside (23) were detected, as reported before, togather with the newly found secologanoside 11-methyl ester (34), the structure of which was inferred from the appearance of MS peaks B_1 (*m/e* 139), C (*m/e* 297) and E (*m/e* 165) (Fig. 12). Final confirmation of the structure was carried out on the isolated substance, which will be reported elsewhere.

Several indole alkaloids have been isolated from the plants of the genus

* Although the occurrence of raffinose in this plant has not been reported, the peak was first inferred to have originated from an oligosaccharide by GC–MS and the substance was finally identified by comparison with an authentic sample.

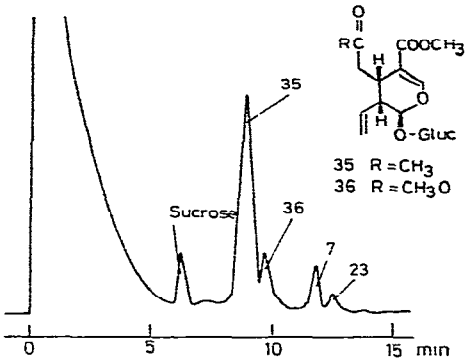

Fig. 14. Chromatogram of the extract of *Adina racemosa* after methylation on a 1.5% OV-1 column (1.8 m) at 270°.

Adina[23–32]. The present examination of *A. pilulifera* showed the occurrence of morroniside (22) as the sole iridoid glucoside (Fig. 13). However, the examination of *A. racemosa* further revealed the presence of several glucosides. Conventional GC on OV-17 of the extract of this plant showed peaks corresponding to secologanin (21) and/or loganin (7), secologanoside 11-methyl ester (34) and sweroside (23). However, GC of the extract on OV-1 column, after methylation with diazomethane in methanol followed by trimethylsilylation, showed peaks of the four glucosides loganin (7), sweroside (23), the 7-keto derivative (35) of secologanin and secologanoside 7,11-dimethyl ester (36), indicating the presence of the four glucosides described above, namely loganin (7), secologanin (21), sweroside (23) and secologanoside 11-methyl ester (34), in the plant (Fig. 14). The appearance of peaks C (*m/e* 223) and E (*m/e* 165) in the spectra (70 eV) of glucoside (35) indicated its secoiridoid structure. Even at 17 eV, this compound showed peak E with considerable intensity together with peaks C and D. The facile formation of the 7-keto derivative (35) of secologanin on methylation of the glucoside (21) has already been reported[33]. The absence of a peak due to compound (21) in the chromatogram of the methylated substances also indicates the structure. With the 7,11-dimethyl ester (36), the GC peak was too weak for GC–MS to be performed. However, the appearance of the peak of 11-methyl ester (34) on OV-17 readily rationalized the formation of the dimethyl ester (36). Both the 7-keto derivative (35) of secologanin and secologanoside dimethyl ester (36) were further identified with authentic samples by GC comparisons.

As *Allamanda cathartica* (Apocynaceae) belongs to the sub-family Plumerioideae, the occurrence of iridoids in this plant was to be expected. GC on OV-17 of the extract of this plant indicated peaks as shown in Fig. 15*. On GC–MS (70 eV), the species with a relative retention time of 6.70 gave peak B_1 (*m/e* 139), together with several peaks that were due to the sugar moiety at *m/e* 361, 271, 217, etc. Accordingly, this substance was assumed to be an iridoid glucoside. On the other hand, at 17 eV, it showed prominent peaks at *m/e* 452, 363 and 273. The differences among the three peaks are in accordance with 89 = TMS-O and 90 = TMS-OH, respec-

* By the same means as for *P. scandens*, it was found that the substance with a peak with a relative retention time of 13.07 is raffinose.

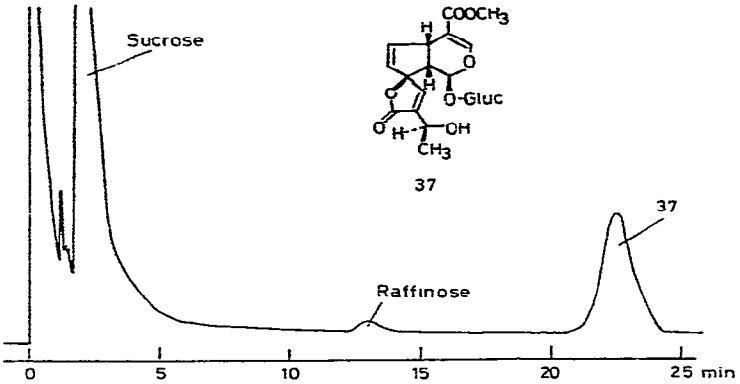

Fig. 15. Chromatogram of the extract of *Allamanda cathartica* on a 1.5% OV-17 column (1.8 m) at 280°.

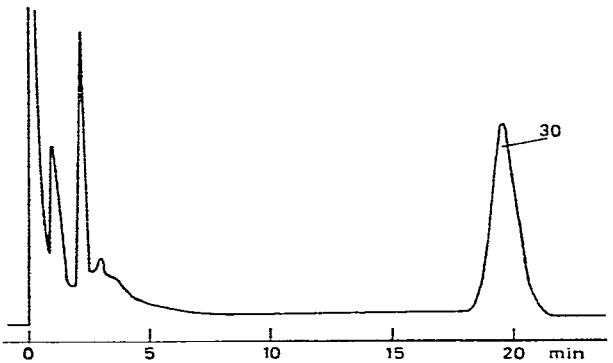

Fig. 16. Chromatogram of the extract of *Osmanthus heterophyllus* on a 1.5% OV-17 column (0.5 m) maintained for 5 min at 250° and then temperature programmed from 250 to 300° at 4°/min.

tively. The difference of *m/e* 90 also implies the presence of a hydroxyl group in the aglucone moiety. These three peaks were also observed at the measurement at 70 eV, although the intensities were weak. Assuming the original glucoside to be plumieride, the appearance of these peaks could be reasonably explained. Actually, this glucoside was identified as plumieride (37) by GC*. Although the relative retention time of compound (37) on OV-17 was the same as that of asperuloside (18), the former had a value of 4.00 on OV-1 under the conditions given in Table I.

Ligustroside (30) was detected in *Osmanthus heterophyllus* (Fig. 16).

For the plants *A. pilulifera* etc. whose iridoid constituents were first examined, all of the glucosides detected by GC were actually isolated and identified, and the results will be reported elsewhere.

ACKNOWLEDGEMENTS

Plumieride and hastatoside were donated by Prof. H. Schmid of Zurich Uni-

* During the course of this work, a report on the isolation of plumericin, etc., from *A. cathartica* was published[34], but plumieride (37) was not isolated.

versity and Prof. H. Rimpler of Freie University, Berlin, respectively, to whom the authors express their gratitude. Thanks are also due to Prof. H. Mitsuhashi of Hokkaido University, Dr. H. Murata at Ibusuki and Dr. M. Goto of Kyoto Herbal Garden, Takeda Chemical Industries Ltd., for their help in collecting the plant materials.

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