The Thai Journal of Pharmaceutical Sciences

Volume 11
Issue 4 *1986*

Article 1

1-1-1986

CHEMICAL CONSTITUENTS IN THE LEAVES OF ABRUS PRECATORIUS L.(สารเคมีจากใบมะกล่ำตาหนู)

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ORIGINAL ARTICLE

CHEMICAL CONSTITUENTS IN THE LEAVES OF *ABRUS PRECATORIUS* L.

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ABSTRACT

The leaves of *Abrus precatorius* Linn. were extracted with methanol and their chemical substances were separated by using chromatographic and crystallization techniques. A flavanone glycoside was isolated and identified as hemiphloin or 6-C-glucosylnaringenin which has never been reported to be found in this species or family before. Another chemical substance isolated from the hydrolysed crude extracts was proved to be triterpenoid sapogenin, olean-12-ene type, named abruslactone A. This triterpenoid isolated from the roots and vines of *Abrus precatorius* Linn. has been reported.

(Th. J. Pharm. Sci. 11(4) 197-203 (1986))

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KEY WORDS

Abrus precatorius Linn., Leguminosae, 6-C-glucosylnaringenin, hemiphloin, triterpenoid sapogenin, olean-12-ene, abruslactone A.

INTRODUCTION

In China, Indonesia and Malaysia, the leaves of *Abrus precatorius* Linn. has been used to treat conjunctivitis, hoarseness, swollen tonsils and sprue. (1)

There are many kinds of substances being isolated from *Abrus* species such as flavonoids, triterpenoid saponins, alkaloids, proteins, and polysaccharides. Bhardwaj *et al* (2) reported the isolation of flavonoids; luteolin, abrectorin, orientin and desmethoxycentaureidin 7-0-rutinoside from *Abrus precatorius* Linn. seed kernels. Lupi *et al* (3) also isolated abruquinones, the isoflavanquinone from the root of the same plant. For triterpenoid saponins, Chiang *et al* (4) isolated abruslactone A, methylabrusgenate and abrusgenic acid from the roots and vines.

This is the first report of 6-C-glucosylnaringenin or hemiphloin and the C-glycosylflavanone in the genus *Abrus*. The ^{13}C-NMR spectrum data of hemiphloin and abruslactone A are reported.

EXPERIMENTAL

Source of material. The leaves of *A. precatorius* L. were collected from Nakorn Sawan, Thailand. The plant sample is identical with the Herbarium of Royal Forest Department, Bangkok.

Melting points were determined on a Reichert heating stage microscope and are uncorrected. IR spectra were measured on a Shimadzu-440 using KBr disc. NMR (^{1}H and ^{13}C) spectra were recorded with a Jeol FX-900 in acetone-d_6 + $CDCl_3$, CD_3OD, $CDCl_3$ solutions with TMS as internal reference. Mass spectra were obtained on a Jeol DX-300/JMA 2000 operating at 70 eV.

EXTRACTION AND ISOLATION

One kilogram of the dried leaves was extracted in a Soxhlet apparatus with methanol for 24 hours. After evaporation of solvent under reduced pressure, the extract was suspended in 100 ml water and extracted thoroughly with chloroform (4 × 200 ml.). The aqueous layer was concentrated to give 92 grams of Extract A which was separately treated in two ways. One (42 g) was isolated by column chromatography and the other (50 g) was hydrolysed, extracted and isolated.

Only 42 grams of Extract A was isolated by quick column chromatography (silica gel G/ chloroform; chloroform: methanol 95:5, 90:10, 85:15, 80:20, 70:30, 60:40, 50:50; methanol and water). The eluted fractions with chloroform : methanol 85:15, 80:20 and 70:30 were combined and evaporated to dryness to give Extract B (11 g) containing at least 3 components as detected by TLC. The Extract B was further chromatographed over silica gel and eluted with chloroform : methanol 8:2 to give white-cream needle crystal of a flavonoid glycoside, named Compound A (35 mg.).

The remaining 50 grams of Extract A was dissolved in water and extracted with n-butanol (4 × 200 ml). The extract was evaporated to dryness before dissolved in 200 ml methanol and mixed with 2N sulfuric acid (200 ml). The mixture was refluxed at 60°C for 10 hours. After cooling, 100 ml ice water

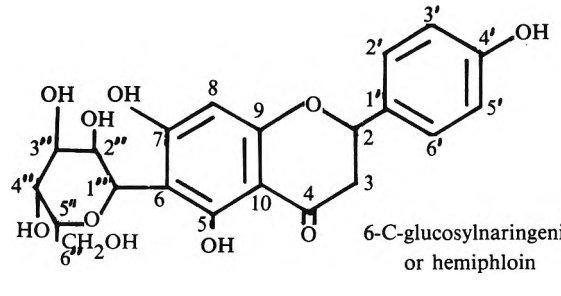
was added and extracted with diethylether (5×200 ml). The ether layer was washed with 5% KOH and water, then concentrated to give crude sapogenin (5 g) containing at least 8 components as detected by TLC. The crude sapogenin was chromatographed (silica gel/chloroform : ethyl acetate 8:2), 9 fractions were received and the first fraction was rechromatographed (silica gel/chloroform) to give white needle or rod crystal of triterpenoid sapogenin, named Compound B (100 mg).

COMPOUND A white-cream needle crystal from methanol, mp. 108-9°C (uncorrected), $C_{21}H_{22}O_{10}$, TLC : polyamide (Woelm) : $CHCl_3$-MeOH-EtCOMe 9-4-2 (0.27), silica gel G (E. Merck) : $CHCl_3$-MeOH 7-3 (0.20), UV : λmax (methanol) 227, 292, 330 (sh) nm, IR : (KBr) νmax (cm^{-1}) = 3350(OH), 2880, 1655 (C=O), 1618, 1520, 1465 (C=C), 1350, 1230, 1030 (C-O-C), 1176, 1410, 1310, 1084, 800, 760, ^{1}H-NMR : (acetone-d_6 + $CDCl_3$, 250 mHz) δ 2.80 (2H, q), 3.15 (2H, q), 3.45-3.90 (6H, m, glucosyl), 4.90 (1H, d, H-C1″), 5.50 (1H, dd), 5.96 (1H, s, H-C8), 6.89 (2H, d, H-C3′, H-C5′), 7.38 (2H, d, H-C2′, H-C6′), 7.94 (1H, s, OH-C5), 8.92 (1H, s, OH-C7) ^{13}C-NMR : (CD_3OD, 90 mHz) δ 44.16 (t, C-3), 63.17 (t, C-6″), 72.06 (C-4″), 72.93 (C-1″), 75.42 (C-2″), 80.41 (d, C-2), 80.87 (C-5″), 96.96 (C-8), 103.54 (C-10), 106.19 (C-6), 116.6 (C-3′, C-5′), 129.33 (C-2′, C-6′), 131.17 (C-1′), 159.23 (C-4′), 164.43 (C-5), 167.53 (C-7), 198.3 (C-4=0) MS : 70 eV, m/e (rel. int. %) 434 (0), 416 (57), 398 (11), 380 (7), 286 (25), 285 (42), 272 (40), 165 (100), 153 (21), 152 (24), 120 (65), 91 (19), 69 (28), 55 (22).

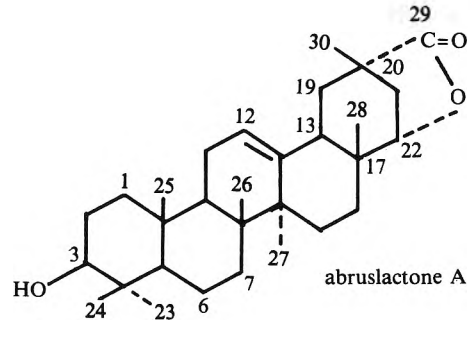
COMPOUND B white needle or rod crystal from chloroform, mp. 323°C (uncorrected), $C_{30}H_{46}O_3$, TLC : silica gel G (E. Merck) : chloroform-ethyl acetate 8-2 (0.64), silica gel G (E. Merck) : benzene-diethylether 6-4 (0.46), silica gel G (E. Merck) : chloroform-methanol 7-3 (0.91), UV : λ max (methanol) = 239 nm IR : (KBr) ν max (cm^{-1}) = 3500 (OH), 2970, 1750 (C=O), 1635 (C=C), 1455, 1390, 1365, 1170, 1138 (C-C(=O)-O), 1100 (O-C-C), 1040, 1014, 955, ^{1}H-NMR : ($CDCl_3$, 90 mHz) δ 15.66 (m, C-24, C-25), 17.01 (q, C-26), 18.31 (t, C-6), 20.97 (t, C-11), 23.51 (t, C-16), 24.06 (q, C-30), 24.33 (q, C-28), 24.98 (q, C-27), 25.19 (t, C-15), 27.2 (t, C-2), 28.12 (q, C-23), 33.16 (t, C-7), 33.86 (t, C-21), 35.27 (s, C-17), 37.01 (s, C-10), 38.63 (t, C-1), 38.74 (s, C-4), 39.34 (s, C-8), 39.5 (s, C-14), 39.88 (d, C-18), 42.53 (s, C-20), 43.45 (t, C-19), 47.52 (d, C-9), 55.21 (d, C-5), 78.89 (d, C-3), 83.06 (d, C-22), 124.62 (d, C-12), 140.22 (s, C-13), 182.32 (s, C-29), MS : 70 eV, m/e (rel. int. %), 454 (M^+, 2), 436 (4), 246 (100), 231 (9), 228 (11), 218 (16), 207 (20), 201 (15).

RESULTS

Compound A and B are identified as hemiphloin and abruslactone A, respectively.



6-C-glucosylnaringenin or hemiphloin



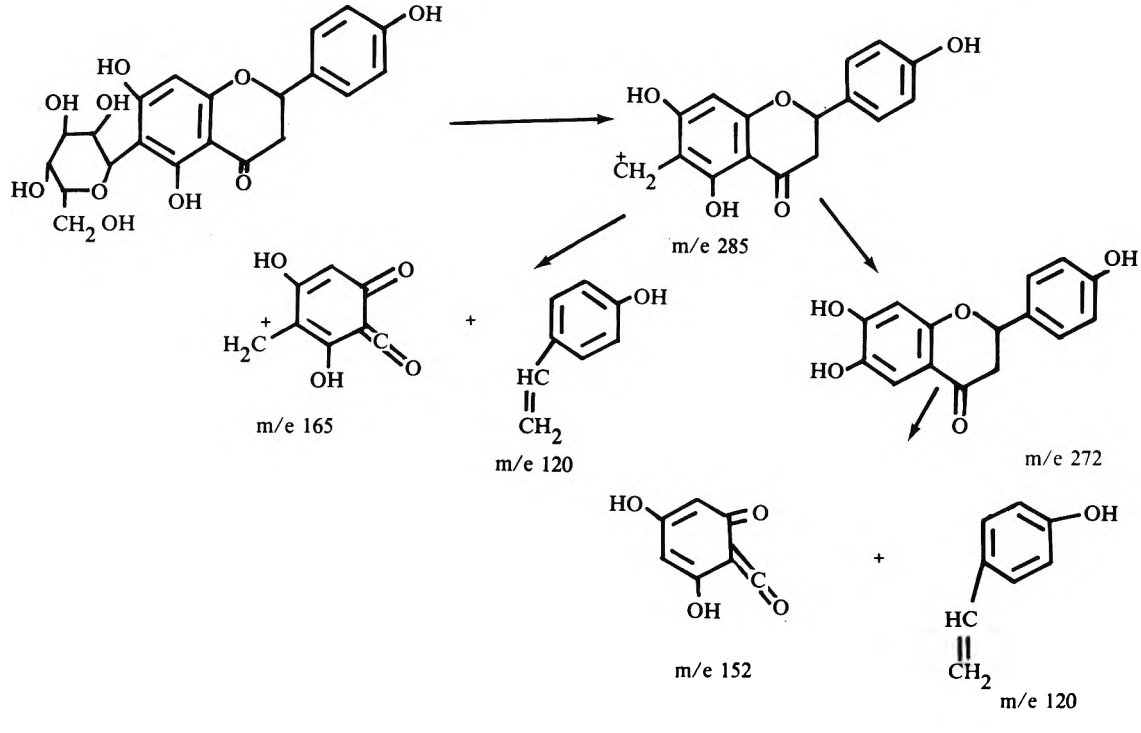
abruslactone A

Discussion and Conclusion

The isolated Compound A gave positive test with Mg + HCl (pink to red) and $AlCl_3$ (fluoresce green). The UV absorption spectrum was typical for flavanone (5). The ^{1}H-NMR spectrum in acetone-d_6 and $CDCl_3$ showed the presence of two geminal protons of C-3 which appeared as 2 sets of doublet of a doublet at 3.15 and 2.80 ppm. The difference of the resonating signals between the two protons was probably due to the anisotopic effect of the carbonyl function. The splitting pattern of the mention protons indicated that they also coupled with a nearby C-2 proton at 5.5 ppm. This confirmed that Compound A was a flavanone.

The four (A_2B_2 system) protons of C-2′, C-6′ and C-3′, C-5′ of the ring B were observed at 7.38 and 6.89 ppm. This indicated that the C-4′ was substituted. Only one proton singlet was observed at 5.92 ppm which indicating of five substitutions in the ring A of the flavanone.

The ^{13}C-NMR spectrum in CD_3OD showed the signal of C-1″of glucose at 72.93 ppm which indicated that the sugar was attached to the flavonoid nucleus by C-C linkage (O-C linkage, the signal would be at 101 ppm) (6). The triplet at 44.16 ppm and a doublet at 80.41 ppm indicated the presence of a methylene C-3 and a methine C-2 functions respectively. the signals of C-5, C-7 and C-4′ were corresponding to those signals of the naringenin (7). For C-6, the signal was at 106.19 ppm showed that the substituent group was not hydroxyl (140 ppm) nor methoxyl (130-150 ppm) but was closed to the C-glucosyl (108 ppm). The EIMS spectrum of underivatized compound showed three peaks due to the loss of three molecules of water (416, 398, 380) and the ionization of the Compound A (6) was as the figure below.



Besides the evidences from the ^{1}H-NMR and ^{13}C-NMR spectra, the structure determination of this compound as 6-C-glucosylnaringenin or hemiphloin was also based on TLC, mmp, IR and MS spectra comparison under the same condition with authentic hemiphloin which was isolated by Budzianowski and Skrzypczakowa (8) from *Tulipa gesneriana* cv "Paradae" (Liliaceae).

The isolated Compound B gave positive test with Liebermann Burchard test (pink) which classified the compound roughly as triterpenoid (9). The IR spectrum showed a hydroxy band at 3500 cm^{-1} and a band at 1750 cm^{-1} attributable to a lactone carbonyl group. The nature of the three oxygen functions in the molecule was thus established. The ^{1}H-NMR spectrum showed signals corresponding to seven methyl groups bonded to quarternary carbon atoms which indicating the presence of a β-amyrin skeleton (10). From ^{13}C-NMR spectrum, more details of Compound B are shown, there are double bond at C-12 and C-13 (124.62 and 140.22 ppm., respectively). The lactone group (C-29) attached to C-20 and C-22 of ring E (43.45 for C-20, 83.06 for C-22-0 and 182.32 for  -29-0). A hydroxyl group attached to ring A at C-3 (78.89 ppm) and seven methyl groups bonded to quarternary carbon atoms (15.66, 15.66, 17.01, 24.06, 24.33, 24.98 and 28.12 ppm) (11, 12, 13). The mass spectrum clearly revealed that the compound belongs to the olean-12-ene type. From the fragmentation pattern (m/z 246, the base peak and 207, typical of the retro-Diels-Alder breakdown), it was evident that the lactone group is located in ring D or E and the hydroxyl group is attached to ring A or B (10). The structure identification of Compound B as abruslactone A was mainly based on IR, ^{1}H-NMR, ^{13}C-NMR and MS spectra comparison with spectral data of the isolated abruslactone A by Chiang *et al* (4).

ACKNOWLEDGEMENT

The authors are sincerely grateful to Dr. Budzianowski, Institute of Biology and Pharmacy, Medical Academy, Poznan, Poland, for donating the authentic sample of hemiphloin. Our grateful thanks are also due to Chulalongkorn University for granting Rachadapiseksompoj Research Fund to conduct this work.

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สารเคมีจากใบมะกล่ำตาหนู

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บทคัดย่อ

จากการสกัดใบมะกล่ำตาหนู (*Abrus precatorius* Linn.) ด้วยสารละลายเมทานอล และนำมาแยกสารสำคัญ โดยอาศัยเทคนิคทางรงคเลข และการตกผลึก จนได้สารบริสุทธิ์พวก flavonoid glycoside ชนิด flavanone ที่ชื่อ hemiphloin หรือ 6-C-glucosylnaringenin ซึ่งไม่เคยพบในพืชชนิดนี้ หรือวงศ์นี้มาก่อน นอกจากนี้เมื่อนำสารสกัดรวมของใบมะกล่ำตาหนูมาไฮโดรไลซ์ด้วยกรด สามารถแยก triterpenoid sapogenin ชนิด olean-12-ene ที่ชื่อ abruslactone A สารนี้ได้มีผู้รายงานว่า พบในรากและเถาของต้นมะกล่ำตาหนูมาก่อน

(ไทยเภสัชสาร 11 (4) 197-203 (2529))

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