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Phytochemical Investigation of the Leaves of Paeonia decora Anders.

Paeonia decora Anders. Yapraklarının Fitokimyasal Tetkiki

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In a previous paper(1), it was reported that no work was done with *P. decora* Anders. According to a new survey in the literature, it was found that there is one paper on the roots of *P. decora*(2), but no work was done with its leaves.

The ethanolic extract of the leaves had an activity ** ED_{50} 3.5×0 against cell culture 9KB test system(3).

The aim of this study was to find the active compounds of the plant as well as the other compounds.

EXPERIMENTAL

500 g of the air dried leaves of the plant were macerated, then percolated with a 50% aqueous solution of ethanol. The extract was evaporated under a vacuum to dryness. The residue (50 g) was suspended in water and extracted with ethyl acetate. The remaining aqueous solution, upon evaporation to a small volume under a vacuum, yielded a yellow precipitate. Two flavonol glycosides were found in this precipitate. The details of their isolation and structural work will be published soon as a separate paper.

Fractionation of the ethyl acetate extract: The EtOAc extract was evaporated under reduced pressure to dryness. The residue dissolved in aqueous ethanol and made alkaline with NaOH, then extracted with $CHCl_3$. Thus 12.4 g of a green residue was obtained. This

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was chromatographied in an Al_2O_3 (activity III) column (3×50 cm) using the following solvents :

Petroleum ether
Petroleum ether-benzene (7 : 3)
Petroleum ether-benzene (1 : 1)
Benzene
Benzene-chloroform (1 : 1)
Chloroform
Chloroform-ethanol (1 : 1)
Ethanol

Each fraction was checked on thin layer and those which gave the same spots were combined. The results of this fractionation were shown on Table I.

Table I. Column chromatographic separation of the green residue.

Frac. No.	Eluent	Combined frac. No.	Frac. wt. mg	Isolated comp.	Yield mg.
1 - 6	Petroleum ether	1 - 2	150	$C_{35}H_{72}$	30
7 - 12	Pet. ether - benzen (7 : 3)	3 - 20	1500	$C_{34}H_{70}O$	100
13 - 28	Pet. ether - benzene (1 : 1)				
29 - 40	Benzene				
41 - 54	Benzene - chloroform (1: 1)	21 - 50	300	$C_{29}H_{50}O$	150
55 - 60	Chloroform				
61 - 65	Chloroform - ethanol (1 : 1)				
66 - 70	Ethanol	51 - 70	A group of substances which were not separated		

Isolation and identification of n-pentatriacontan ($C_{35}H_{72}$) :

Fractions 1 and 2 from Al_2O_3 column yielded a white amorphous material, thin layer chromatography on three different solvent sys-

tems showed that it was a single compound with minor impurities. The compound was crystallized from MeOH : $CHCl_3$ (8 : 2) and from n-hexane. Shiny plates were obtained, m.p. was 73 - 74°C (literature 74.4 - 74.6°C) (4), optical rotation (α) $D^{22} = \pm O°$ (in $CHCl_3$), the compound did not exhibit UV spectra, IR spectra showed typical bands of a long chain normal hydrocarbon without hydroxyl or carbonyl bands at 2950, 2860, 1470, 1370, 725 and 715 cm^{-1}. NMR curve gave two end methyl groups at 0.85 ppm and the long chain methylene groups were at 1.25 ppm (δ), the integration gave 6 protons for methyl and 66 hydrogens for methylene groups. Analytical findings (Found : C, 86.00; H, 14.0, calculated for $C_{35}H_{72}$: C, 85.36; H. 14.63%).

Isolation and characterisation of n-tetratriacontanol ($C_{34}H_{70}O$):

Fractions 3 - 20 yielded 1.5 g of an orange color mixture. Thin layer showed one major spot with a group of other spots. Crystallization from different solvents did not give a clean single compound. The product was chromatographied in Al_2O_3 column (1×30 cm). The column was eluted with petroleum ether then with benzene. The first two fractions of benzene yielded shiny plates. Upon recrystallization from methanol, a single compound was obtained, m.p. was 90 - 91°C (literature 92 - 92.2°C) (5), optical rotation (α) $D^{22} = \pm O°$ (in $CHCl_3$). No UV maxima was obtained, IR curve gave a long chain normal hydrocarbon bands at 3370, 2970, 2850, 1450, 1370, 1050, 725 and 715 cm^{-1}. NMR spectra gave an OH band at 0.82 ppm, this was correlated by deutron exchange method, the end methyl group was at 0.95 ppm, the long chain methylene groups were at 1.25 ppm and the methylene group next to OH group was at 3.55 ppm (δ). The integration of the spectrum showed one proton for OH, three protons for CH_3, 64 protons for $(-CH_2-)_{32}$ and 2 protons for $-CH_2-O-$ groups. Analytical findings (Found : C, 81.85; H, 14.8, calculated for $C_{34}H_{70}O$: C, 82.59; H, 14.17%).

Isolation and identification of β-sitosterol :

Fractions 21 - 50 yielded 300 mg of a green residue. Thin layer chromatography showed a single compound, with some impurities, alcohol crystallization yielded 150 mg of a white compound, m.p. was 137°C (literature 137°C) (6), optical rotation (α) $D^{22} = -35.2$ (in $CHCl_3$). Anal. calc. for $C_{29}H_{50}O$: C, 84.05; H, 12.07 found C, 83.9; H, 12.50% UV

curve gave a shoulder at 205 nm (an isolated double band). IR curve showed the typical bands of a steroidal alcohol. Mixed m.p.'s, the IR curve and the thin layer comparison with the standard sample, proved that this was β-sitosterol.

This could be the active part of the plant as well as the flavonol glycosides.

Aqueous alkaline solution :

The alkaline solution left from the chloroform extraction was made neutral with dilute hydrochloric acid. This solution was chromatographied using paper and thin layer; when the chromatograms were sprayed with a 5% solution of $FeCl_3$, blue-blue gray streaks were obtained. Standard sample comparison showed the presence of gallic acid. In order to obtain the free gallic acid, the solution was evaporated to dryness, then extracted with a saturated solution of Na_2CO_3. When Na_2CO_3 solution was acidified, a pale colored product was obtained. Ethanol crystallization gave a compound with a m.p. 240°C. Mixed m.p.'s and IR curve comparison proved that this was gallic acid.

SUMMARY

The ethanolic extract of the leaves of *Paeonia decora* Anders. had shown potential antitumor activity against the 9KB test system. Fractionation of the ethanolic extract yielded two flavonal glycosides, and β-sitosterol, n-pentatriacontan ($C_{35}H_{72}$), n-tetratriacontanol ($C_{34}H_{70}O$) and gallic acid. β-sitosterol and the flavonol glycosides could be the active principles of the plant.

ÖZET

Paeonia decora Anders. yapraklarının etanollü ekstresi 9KB test sisteminde kuvvetli antitümöral aktivite göstermiştir. Etanollü ekstrenin fraksiyonlandırılması ile iki flavonol glikozidinden başka β-sitosterol, n-pentatriakontan, n-tetratriakontanol ve gallik asit elde edilmiştir. β-sitosterol ve flavonol glikozidlerinin, nebatın aktif kısımları olduğu tahmin edilmektedir.

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