# ESSENTIAL OIL FROM GLANDULAR LEAVES OF SOME SPECIES OF *Rosa* L.* **

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Combination of gas chromatography and mass spectrometry were employed for chemical analyses of essential oils from glandular leaves of seven species of *Rosa* L. It was suggested that this approach may serve as one of the additional criteria for botanical systematics.

Some original species of the genus *Rosa* L. are characterized by the presence of glandular hairs on their axis, leaves and flowers, which contain excretions of typical scents, but which differ from the perfumes of the essential oils from the crown petals. The botanists studying systematics observed that the odours from these small glands are relatively specific and also demonstrated that the odours are characteristic of various sections of the very complex genus *Rosa* L. (*cf.*[1,2]). In connection with the study of natural substances, especially isoprenoids, we endeavoured to characterize chemically the essential oils present in the glandular hairs by more recent separation and identification methods. Our aim was to ascertain to what extent the chemical composition of these essential oils of different odour are a typical characteristic[3-6] utilizable for the classification of the species of this genus.

Earlier literature data on the chemical composition of the essential oils of roses only concern the essential oils obtained from flowers or buds. In this respect the species *Rosa damascena* MILL (*cf.*[7-9]) cultivated in Mediterranean and Black Sea areas has been more thoroughly investigated, due to the fact that it is the source of the long known and technologically produced "rose oil". In another comparative study[10] the chemical composition of the essential oils from several varieties of fraggrant and decorative roses is described. In our laboratories we investigated comparatively in connection with older studies[11] the chemical components of the excretions from leaves, stalks and calyx glands of 14 original species of roses. At that time the samples were obtained by direct scratching off of the glandular hairs of the

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investigated plants, and the comparison was carried out on the basis of the gas chromatograms obtained with single samples (without identification of single components). In addition to the volatile components proper of the essential oils these samples also contained a larger amount of waxy components the importance of which for the determination of differences among species is less important than in the case of essential oils[3-6]. However, the results which we obtained at that time confirmed the very good agreement between the composition of the extractable fractions, identifiable by gas chromatography, and the belonging of the investigated species of the *Rosa* L. genus to the corresponding section or subsection. Fig. 1 may serve as an example of this agreement, which gives the chromatograms of samples from five species of *Rosa* L. from section *Caninae*. The records obtained with *R. albiflora*, *R. gisellae*, *R. inodora* (subsection *Sepiaceae*) are very similar even with respect to the quantity of single components. A good mutual agreement is also observed between the samples from *R. tomentosa* and *R. sherardii* (subsection *Vestitae*), but the comparison of the records of samples from the two subsections demonstrated a substantial difference.

In this paper we analysed only the volatile components of the glandular shoots from seven original species of roses, belonging to 4 botanical sections (Table I). The waxy components, extractable with ether, were separated from the volatile essential oils by steam distillation. The yields of the essential oils obtained (per weight of fresh foliage), varied between 0·05 to 0·001%. The samples were first analysed by gas chromatography alone and then by a combination of gas chromatography and mass spectrometry. Thus the mass spectra of the majority of gas chromatographic peaks were obtained if the separation on the packed column was sufficiently complete. In all instances the essential oils represented complex mixtures composed mainly of monoterpenoids and sesquiterpenoids. For the majority of the separated peaks their identification succeded either completely or at least partially, by comparison of their mass spectra with those of standard terpenoids, which were at our disposal. The results of the analysis of the essential oil from *R. jundzillii* are shown in Table II. Five terpenic hydrocarbons were identified in the oil, then a group of terpenic alcohols with dominant linalool and a not quite resolved peak of *p*-menthadienol; we further identified six sesquiterpenic hydrocarbons, two sesquiterpenic ketones, and lastly a group of five sesquiterpenic alcohols. Dibutyl phthalate in the sample evidently originates from polyvinyl chloride, where it is present as a softener, during the collection and the working up of the material.* The oil contained only a small amount of substances of non-isoprenoid nature.

* Similarly, styrene, which was identified on the basis of its mass spectrum in some samples, originates from the synthetic resins used; the assumption that the source of styrene could possibly be 2-phenylethanol demonstrated in essential oils from roses[10] was eliminated on the basis of gas chromatography and mass spectrometry of authentic 2-phenylethanol.

The number of terpenic hydrocarbons in other essential oils did not exceed five as a rule, while the number of individual sesquiterpenic hydrocarbons was about 10. The essential oils contained approximately 7–8 individual terpenic alcohols (maximum 14 in *R. albiflora*), and 6–7 sesquiterpenic alcohols; their maximum number was found in *R. zalana*, *i.e.* fifteen. Typical terpenic hydrocarbons in the essential oils of the investigated roses were α- and β-pinene and myrcene, while among sesquiterpenic hydrocarbons caryophyllene, bourbonene[12], muurolene[13], cadinenes and humulene occurred most commonly. None of the abundantly present sesquiterpenic alcohols could be identified accurately so far.*

TABLE I
List of the Analysed Original Species of *Rosa* L.

| *Rosa* | Section | Subsection | No of chromosomes |
|---|---|---|---|
| *rubiginosa* L. | *Caninae* | *Rubiginosae* | 35 |
| *zalana* WIES. | *Caninae* | *Rubiginosae* | 35 |
| *albiflora* OPIZ. | *Caninae* | *Sepiaceae* | 42 |
| *tomentosa* SM. | *Caninae* | *Vestitae* | 35 |
| *pendulina* L. | *Cinnamomeae* | | 28 |
| *gallica* L. | *Gallicae* | | 28 |
| *jundzillii* BESS. | *Jundzilliae* | | 42 |

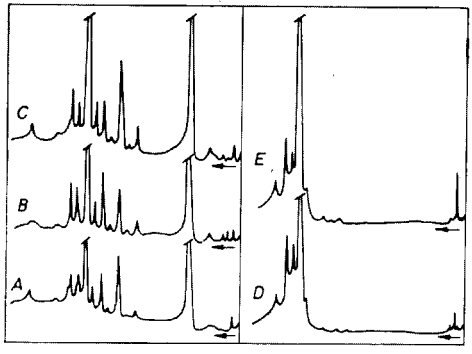


FIG. 1
Chromatograms of the Excretions (essential oils + waxy components) of Glandular Roses
*A R. albiflora*; *B R. gizellae*; *C R. inodora*; *D R. tomentosa*; *E R. sherardii.*

* It is known that the mass spectra of terpenoid substances display small differences[14] and that the experimental conditions and the type of the instrument used also play an important role in the formation of the spectrum. Therefore, the identification of terpenoid substances in this way is rather difficult. Only those substances are considered fully identified the mass spectra of which were identical with those of available and confirmed standards.

A possible contribution of the chemical analysis of the components of the essential oils from roses to the chemosystematics is demonstrated in Fig. 2, where volatile substances of two species of *R. jundzillii* (Table II) and *R. gallica* are compared on schematic GLC-records. The agreement in the composition of both essential oils is very good. They contain twenty substances in common. Peak No 14 in *R. jundzillii* was identified as γ-cadinene, while an equally situated peak in *R. gallica* was β-cubebene[15]. Peaks No 5 and 9 in *R. gallica* were absent, but the sample contained in addition five smaller unidentified peaks (×). The similarity of the chemical composition of the essential oils from both species, of which each belongs to another section (*Gallicae* and *Jundzilliae*), is not surprising, however, because *R. jundzillii* is

TABLE II

Composition of the Essential Oil from *Rosa jundzillii* BESS.

| Number of the peak | Relative retention time[a] | Substance | $M^+$ |
|---|---|---|---|
| 1 | 0·085 | α-pinene | 136 |
| 2 | 0·12 | β-pinene | 136 |
| 3 | 0·125 | ? | |
| 4 | 0·16 | myrcene | 136 |
| 5 | 0·18 | ? | 82 |
| 6 | 0·32 | linalool | 154 |
| 7 | 0·49 | terp. alcohol | 152 |
| 8 | 0·50 | terp. alcohol | 154 |
| 9 | 0·51 | 1,5-*p*-menthadien-7-ol | 152 |
| 10 | 0·70 | ? | |
| 11 | 0·90 | β-bourbonene | 204 |
| 12 | 1·00 | caryophyllene | 204 |
| 13 | 1·05 | α-humulene | 204 |
| 14 | 1·08 | γ-cadinene | 204 |
| 15 | 1·12 | α-muurole | 204 |
| 16 | 1·16 | δ-cadinenene | 204 |
| 17 | 1·19 | sesquit. ketone I | 220 |
| 18 | 1·22 | sesquit. ketone II | 220 |
| 19 | 1·28 | sesquit. alcohol I | 220 |
| 20 | 1·30 | sesquit. alcohol II | 220 |
| 21 | 1·33 | sesquit. alcohol III | 220 |
| 22 | 1·40 | sesquit. alcohol IV | 220 |
| 23 | 1·42 | sesquit. alcohol V | 220 |
| 24 | 1·56 | dibutylphthalate | |

[a] SE-30, programmed temperature 50—140°C, 1°C $min^{-1}$.

considered a hybrid of the original form of *R. gallica* and *R. canina* (or possibly *R. corymbifera*).

We expect to succeed during further investigations in identifying a still greater number of substances in the essential oils of glandular roses and that we shall be able after the working up of a larger number of species to make the still unclear relationships between some species of this taxonomically very difficult genus more precise. The chemical composition of the essential oils from *Rosa* L. seems to be one of the additional criteria for the systematics, valuable especially because the other characters (cytological and morphological) are not always unambiguous for an exact classification.

## EXPERIMENTAL

The samples of various species of roses were collected in June 1973. *R. rubiginosa* and *R. albiflora* were from Tiché údolí near Roztoky (Central Bohemia), *R. zalana* and *R. tomentosa* were obtained from the Průhonice rosarium near Prague. *R. jundzillii* and *R. gallica* were found in the Bohemian Central Mountains near Rýdeč (Northern Bohemia), *R. pendulina* were from Černolice near Prague (Central Bohemia).

*Extraction*: Freshly collected foliage (without flowers) of *R. rubiginosa* (2238 g) was extracted with peroxide-free ether (40 l) at room temperature for 24 hours. Ether was distilled off at normal

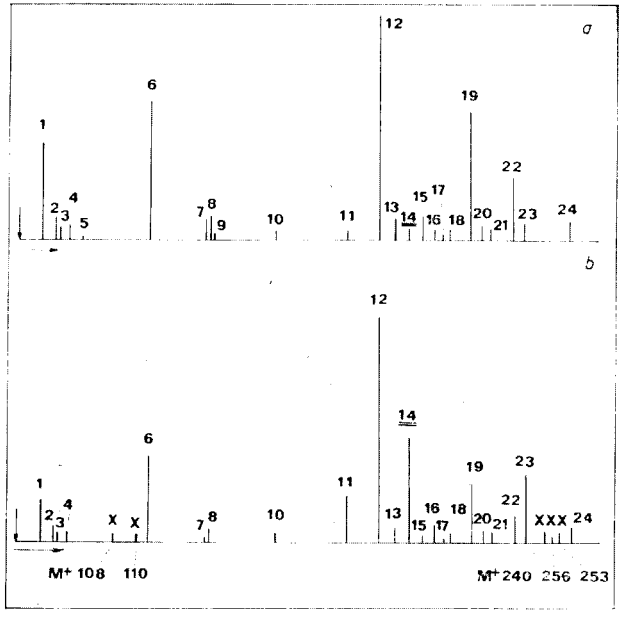


FIG. 2.
Schematic Chromatograms of Two Species of Roses
*a R. jundzillii, b R. gallica.* The numbers correspond to substances in Table II. 3% SE-30, 50°C (10 min), 50 to 140°C (1°C/min). Unidentified peaks are indicated with crosses.

pressure, using a Vigreux column (5 TP). The extract obtained (84 g) was then steam-distilled for one hour. The distillate was collected in an ice-cooled flask containing pentane (30 ml). After a careful evaporation of pentane the residual essential oil weighed 1·1 g (0·05%). The residue in the flask, when worked up, afforded non-volatile waxy components (82 g) which were not investigated so far. The yields of the essential oils of other samples were the following: *R. zalana* 0·002%, *R. albiflora* 0·001%, *R. tomentosa* 0·001%, *R. pendulina* 0·001%, *R. gallica* 0·025%, and *R. jundzillii* 0·018%.

*Gas chromatography* was carried out with a PYE series 104 Chromatograph, Model 64. Preliminary analyses indicated first the optimum conditions for the separation to a maximum number of components. A column packed with 3% SE-30 on Gas Chrom G was found most suitable. The samples were dissolved in tetrachloromethane (1—5% solution) and 0·5 to 2 μl of this solution were injected into the column.

*Mass spectrometry*: The identifications proper were carried out on the same gas chromatograph, connected with a mass spectrometer A.E.I. MS 902, at a programmed temperature interval 50——170°C (ten minutes at the basic temperature, and then a gradient 1°/min) and using a Watson-Biemann separator. The temperature of the ionic source was 100°C and the electron energy 70 eV.

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