ACRIDONE ALKALOIDS OF CALLUS TISSUE OF *Ruta graveolens*

I. N. Kuzovkina, L. D. Kislov,
M. N. Zhivopistseva, Zs. Kozsa,
and K. Szendrei

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A strain of tissue of common rue of root origin (index of the strain R-4) has been obtained which produces up to 3% (of the dry weight) of acridone alkaloids. It has been shown by physicochemical methods that the combined alkaloids consist mainly of 1-hydroxy-N-methylacridone, rutacridone, and 1-hydroxy-3-methoxy N-methylacridone (0.28, 0.31, and 2.41 mg/g dry weight of tissue, respectively). Strain R-4 is a good model for the study of little-known steps in the biogenesis of the acridone alkaloids.

Methods of cultivating plant tissues and cells are widely used at the present time for the study of the biogenesis of natural compounds. It has been established that when plant tissues are grown under artificial conditions the composition of the substances of specialized metabolism synthesized by the cells may change, and the spectrum of these substances does not always coincide with the spectrum of the secondary substances of the whole plant. In view of this, practical interest is presented by those plant tissues which retain the biosynthetic potentialities of the whole plant to the greatest extent.

The work was performed with cultivated tissues and cells of *Ruta graveolens* L. (common rue) and it was shown that the cells of those planted under *in vitro* conditions retain their capacity for biosynthesizing the secondary substances characteristic for the plant — essential oils, coumarins, and alkaloids [1-4]. A change in the composition of the nutrient medium, leading to a change in the amounts of secondary metabolites in the plant cells and selection work with cultivated cells are providing the possibility of isolating, from the callus tissues obtained, lines with a definite direction of their metabolism and with the qualitative composition of the secondary substances that is required for experiment. The lines of cells with different compositions of the secondary substances that are being obtained as the result of selection may serve as a convenient model for the solution of individual questions of the biogenesis of natural compounds.

A characteristic feature of plants of the family Rutaceae, and of common rue, in particular, is their formation of acridone alkaloids, typical representatives of which are 1-hydroxy-N-methylacridone (I) and its methoxylated and isoprenylated derivatives [5, 6].

Tissue cultures obtained from the stem and roots of common rue also retain their capacity for synthesizing and accumulating certain acridone alkaloids in the cells, and by selecting the most strongly colored sections of the tissue on transplantation it is possible to obtain strains of callus tissues possessing considerably higher alkaloid contents than the organs of the intact plant [4, 7].

In the present paper we give the results of a chemical analysis of the acridone alkaloids of a new strain of callus tissue of common rue (index of the strain: R-4).

By chromatographing the benzene-soluble substances of a methanolic extract of R-4 callus tissue, in addition to rutacridone (II) we isolated two other acridone alkaloids. One of them was identified on the basis of UV and IR spectroscopy and its melting point as 1-hydroxy-10-methylacridone (I). The other substance, which dominated quantitatively in the group of acridone alkaloids, likewise had the UV and IR spectra characteristic for acridone alkaloids and

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K. A. Timiryazev Institute of Plant Physiology, Academy of Sciences of the USSR, Moscow. Institute of Pharmacognosy, Medical University of Szeged, Hungarian Academy of Sciences.
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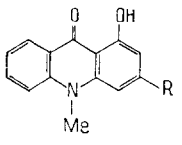
TABLE 1. NMR Characteristics of 1-Hydroxy-3-methoxy-N-methylacridone (III; R=OMe)

Solvent	C_1OH	C_8H	$C_{5,6,7}H$	C_2H	C_4H	NMe	R=OMe
$CDCl_3$ 90 MHz	14.6 (c, 1H)	8.5 (dd, 1H, J=8 Hz)	7.8–7.1 (m, 3H)	6.3 (s, 2H)		3.9 (s, 3H)	3.7 (s, 3H)
CF_3COOD 100 MHz		8.7 (d, 1H, J=8 Hz)	8.4–7.6 (m, 3H)	7.0 (s, 1H)	6.9 (s, 1H)	4.35 (s, 3H)	4.18 (s, 3H)

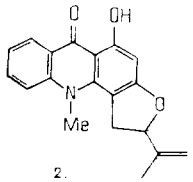
was identified on the basis of its NMR spectrum and melting point as 1-hydroxy-3-methoxy-10-methylacridone (III) (Table 1).

1-Hydroxy-10-methylacridone and also 1-hydroxy-3-methoxy-10-methylacridone, in admixture with another acridone alkaloid — furacridone — have been isolated previously by the authors from the roots of the intact rue plant [8]. The authors were unable to separate the last two compounds and they determined the physicochemical constants of a mixture of these components. However, from the ratio of the integral intensities of the singlets of the N-methyl protons we determined the ratio of the components of the mixture as 1:4 (1-hydroxy-3-methoxy-10-methylacridone:furacridone). Isolation of acridone alkaloids from callus tissue of *Ruta graveolens* of stem origin was reported previously by Scharlemann [9]. On the basis of the results of TLC and UV spectrometry, this author identified in the tissue rutacridone, 1-hydroxy-N-methylacridone, and 1-hydroxy-3-methoxy-N-methylacridone. The quantitative amounts of these alkaloids in the tissue were not reported.



1. R = H

3. R = OMe



2.

Structures of the alkoids isolated: I) 1-hydroxy-N-methylacridone; II) rutacridone; III) 1-hydroxy-3-methoxy-N-methylacridone.

A spectrophotometric determination showed that the total amount of acridone alkaloids in the strain of callus tissue R-4 that we had obtained was about 3% (on the dry weight of the tissue). This is more than 30 times more than the level of acridone alkaloids in the roots of common rue shoots. The predominating compound among the acridone alkaloids of callus tissue R-4 was 1-hydroxy-3-methoxy-N-methylacridone, which made up about 75% of the total amount of acridone alkaloids present.

In this respect, the new strain of callus tissue differs from the strain obtained previously, R-19, for which a predominance of rutacridone was characteristic [7]. The difference in the qualitative compositions of the acridone alkaloids of the two strains of rue callus tissue showed dissimilar directions of the metabolism of this group of alkaloids in them. While in the cells of strain R-19, accumulating mainly rutacridone, the isoprenylation of acridone predominated with subsequent cyclization and the formation of a furan derivative, in the new rue strain R-4 this process was not expressed as completely and the conversion of the usual precursors for the acridone alkaloids stopped at the stage of simpler acridone derivatives — 1-hydroxy-N-methylacridone and 1-hydroxy-3-methoxy-N-methylacridone. In view of the fact that the biogenesis of the acridone alkaloids includes a whole series of unstudied steps, the strain of common rue callus tissue obtained may be considered as a convenient model for the further study of the biosynthesis of alkaloids of this group.

EXPERIMENTAL

Callus tissue R-4 was obtained from the roots of sterile shoots of common rue. The seeds were obtained from the Botanical Gardens of the University of Siena (Italy). The culture was

maintained on agarized Murashige-Skoog medium with the addition of 2,4-D (1 mg/liter) and kinetin (0.2 mg/liter) [10].

For preparative chromatography we used Woelm silica gel, Woelm polyamide, and Sephadex LH-20 (Pharmacia). Analytical chromatography was performed in thin layers of silica gel and polyamide on prepared plates of the Silufol (Kavalier) and Alufolien Polyamid 11 F_{254} (Merck) brands. The chromatographic systems used were hexane–acetone (7:3) (1) and (8:2) (2); benzene–ethyl acetate (8:2) (3); benzene–methanol (9:1) (4); toluene–ethyl acetate–formic acid (5:4:1) (5); ethanol–water (75:25) (6); and petroleum ether–benzene–methanol–water (50:40:5:5) (7). The melting points of the individual substances were determined on a Koffler block; NMR spectra were taken on a Bruker WP 90 instrument, UV spectra on a Specord UV-VIS spectrometer (Carl Zeiss), and IR spectra in paraffin oil and KBr on a UR-10 instrument (Carl Zeiss).

The extraction of the substances from the R-4 callus tissue (weight – 25.4 g of fresh tissue of a 5-week growth) was carried out with boiling methanol in a Soxhlet apparatus. The extracts were evaporated to dryness, and the dry residue was exhaustively extracted with benzene. The yellow fraction of benzene-soluble substances (0.15 g) was chromatographed in a thin layer of silica gel in systems 3 [11, 12], 5 [9], 1, and 4 and in a thin layer of polyamide in systems 6 [12] and 7 [9]. By TLC in comparison with authentic samples and also from the characteristic fluorescences and qualitative reactions with a 3% solution of $FeCl_3$, 1-hydroxy-N-methylacridone (I), rutacridone (II), and 1-hydroxy-3-methoxy-N-methylacridone (III) were identified as the main components in the fractions.

Spectrophotometric Determination of the Amount of Acridones on the Tissue. Part of the total enzyme-soluble substances (corresponding to 0.58 g of the dry weight of the tissue) was chromatographed in a thin layer of silica gel with a combination of systems 2 and 3. The yellow zones of sorbent corresponding to substances (I), (II), and (III) were eluted with methanol and the eluates were used for spectrophotometric determinations in terms of absorption at 400 nm. The concentrations of the alkaloids were calculated from the coefficient of specific adsorption of rutacridone [13]. The amounts of (I), (II), and (III) were 0.28 and 0.81, and 2.41 mg/g of dry weight of tissue, respectively.

Isolation of Individual Substances. On similar treatment, another sample of tissue (16.6 g dry weight) gave 0.11 g of benzene-soluble substances. The combined acridones (I), (II), and (III) were separated with the aid of preparative chromatography on silica gel (system IV), and were then passed through a column of polyamide with gradient elution by mixtures of water and methanol [12]. A mixture of (I) and (II) was extracted from the column with 60% methanol. Compounds (I) and (II) were isolated from the mixture with the aid of preparative chromatography (silica gel, system 1; polyamide, system 7). Elution with 80% methanol gave a fraction from which compound (III) was isolated by means of TLC (silica gel, system 3) and CC (Sephadex LH-20, elution with acetone).

1-Hydroxy-N-methylacridone (I), yellow needles from hexane–acetone, mp 181-189°C (according to the literature: 191-193°C [11], 192-194°C [14]; λ_{max} in ethanol: 244, 301, 313, and 407 nm; ν_{max}. 2850-2970, 1620, 1590, 1460, 1373, 1305, 1280, 1195, 1180 cm^{-1}.

Rutacridone (II), yellow needles from ethanol with mp 160-162°C (according to the literature. 162-164°C [14], 161-162°C [15]); λ_{max} in ethanol: 225, 245, 264, 273, 300, 330, and 400 nm; ν_{max}: 2850-2970, 1630, 1590, 1550, 1460, 1373, and 1325 cm^{-1}.

1-Hydroxy-3-methoxy-N-methylacridone (III), yellow prismatic crystals from acetone with mp 173.5-174 °C (according to the literature 174-176°C [16]); $_{max}$ in ethanol: 225, 249, 264, 275, 300, 330, 400 nm; $_{max}$, 2850-2980, 1620, 1585, 1565, 1510, 1460, 1375, 1320, 1275, 1230, 1210, 1170, 1150 cm^{-1}

SUMMARY

1. A strain of callus tissue has been obtained from common rue which produces considerable amounts of acridone alkaloids – 3% on the dry weight of the tissue.

2. 1-Hydroxy-3-methoxy-N-methylacridone, rutacridone, 1-hydroxy-N-methylacridone have been isolated and identified as the main acridone alkaloids from strain R-4 tissue. The yields of these alkaloids amounted to 2.41, 0.81, and 0.28 mg/g of dry weight of the tissue, their relative amounts being 9:3:1.

3. The strain of common rue callus tissue obtained is of great interest as a model of the study of details of the biogenesis of the acridone alkaloids.

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VARIETY CHARACTERISTICS OF SOYBEAN SEEDS IN RELATION TO PROTEIN AND OIL CONTENTS AND ACTIVITIES OF PROTEINASE INHIBITORS

N. Nigmonov and V. A. Shibnev

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The protein and oil contents and the activity of proteinase inhibitors in six varieties of soybean have been studied. It has been found that the specific amidase activity of trypsin inhibitors ranges from 170 to 320 nominal units. Electrophoretic results indicates the presence in the water-soluble fraction of seven or eight components possessing inhibitor activity in relation to trypsin and chymotrypsin.

Proteins capable of suppressing the activity of a number of proteinases of animals and microorganisms have been found in soybean seeds [1-3]. Although these protein inhibitors are being studied intensively their functions in plants have so far remained obscure.

In the present paper we give the results of a comparative investigation of the activities of trypsin and chymotrypsin inhibitors in the seeds of a number of varieties of the soybean. The characteristics of protein and oil contents and the ratio of trypsin inhibitors to chymotrypsin inhibitors are given. Since the proteinase inhibitor proteins consist of a combination of protein, we have analyzed their component compositions with the aid of electrophoresis. In addition, we have attempted to evaluate the influence of geographical-ecological factors on the activity of the protein inhibitors in the soybean varieties studied.

As can be seen from Table 1, the protein content of the seeds ranged from 44.4 to 37.8%. The amplitude of variability was ±6.6%. The highest amount of protein was found in the variety Rannyaya-10 (44.4%), and the lowest in the variety Éra (37.8%).

Institute of Plant Physiology and Biophysics, Academy of Sciences of the Tadzhik SSR, Dushanbe. Institute of Molecular Biology, Academy of Sciences of the USSR, Moscow.

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