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A CHEMOTAXONOMIC STUDY ON RUSSIAN FAR-EASTERN CAMPANULACEAE

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Abstract-Sterols and triterpenes contained in the roots of 18 species belonging to 6 genera of Far-Eastern Campanulaceae have been examined. ***Astrocodon expansus was*** considered as being separate from the genus ***Campanula.*** Of all the Far-Eastern genera, only ***Peracarpa was*** not studied. The species of ***Codonopsis, Adenophora, Campanula, Asyneuma*** and ***Astrocodon*** studied in the present work contain the same sterol. ***Codonopsis*** is most conspicuous for sterol and triterpene content. The ***Adenophora*** species have the same sterol composition, and ***Campanula langsdorffiana,*** which belongs to the ***Heterophylla*** subsection, is outstanding in ***Campanula. Adenophoru remotiflora,*** recently assigned to a special section, differs from the other species belonging to the same genus in that it contains substances present in ***Campanula, Asyneuma*** and ***Astrocodon.***

INTRODUCTION

RESEARCH on the chemical systematics of Campanulaceae[1,2] and studies on the composition of its species[3] were probably stimulated by the wide use of the family in Oriental medicine,[4] as well as by the necessity for aiding taxonomy by the use of independent objective methods.

Available data on the chemical composition of various Campanulaceae are still insufficient to make a final conclusion on the most important substances which may be used as independent biochemical characteristics. With chemotaxonomic analysis in view, Erdtman[5] suggested the use of compounds which do not take part in the main process of metabolism and are not sensitive to the effects of external factors. Triterpenoids and steroids may be considered as such secondary products. Ponsinet and Ourisson[6] showed the possibility of using triterpenoids of the tetracyclic series in a chemotaxonomic study of 88 ***Euphorbia*** species. They noted that the distribution of these compounds in ***Euphorbia*** are in agreement with the systematic proposals based on morphology.

Using numerous plants, Tsukamoto[7] showed that the distribution of triterpene alcohols and sterols does not depend on the habitat, and is essentially a stable chemotaxonomical feature. At the same time, there are data indicating the influence of environment on the synthesis of triterpenoids.[8] As for sterols, which are present in all higher plants, it should be noted that they may be used for characterizing taxa larger than species, namely genus, family and order.

[1] R. HEGNAUER, ***Chemotaxonomie der Pflanzen.***, Band. 1-4 Basel-Stuttgart (1962-1966).
[2] R. E. ALSTON and B. L. TURNER, ***Biochemical Systematics, New*** Jersey (1963).
[3] T. AKNAMA, 0. TANAKA and S. SHIBATA, ***Chem. Pharm. Bull.*** **16**, 2300 ***(1968).***
[4] J. ROI, ***Traité des plantes medicinales chinoises,*** Encyclopédie biologique, Paris (1955).
[5] H. ERDTMAN, ***Perspectives in Organic Chemistry*** (edited by A. R. TODD), p. ***320,*** Translated from English. Moscow (1959).
[6] G. PONSINET and G. OURISSON, ***Adansonia 8, 227 (1968).***
[7] T. TSUKAMOTO, A. GAGI, K. MIHASHI and J. MORI, ***Chem. Pharm. Bull.*** **66, 2123** ***(1968).***
[8] L. G. MARTJUCHINA, ***Zh. Obschei Khimii 34, 8 (1964).***

In this work we have attempted to use qualitative and approximate quantitative data on the composition of triterpenoids, sterols and other compounds that react with the Carr–Price reagent for steroid compounds,[9] in order to carry out a chemotaxonomic analysis of the Far-Eastern Campanulaceae. The genera *Codonopsis*, *Platycodon*, *Adenophora*, *Asyneuma* and *Campanula* have separated morphologically in the process of development, and are now recognized as independent and well-separated branches of the family 'evolutionary tree'.

TLC[10] with authentic samples was used as the principal method of analysis. The exact chemical identification of the substances contained in the plants studied was not the object of the present work.* The R_f value on TLC is quite sufficient to assign the compounds to a definite class and to examine the difference in chemical composition of the roots of the different species.

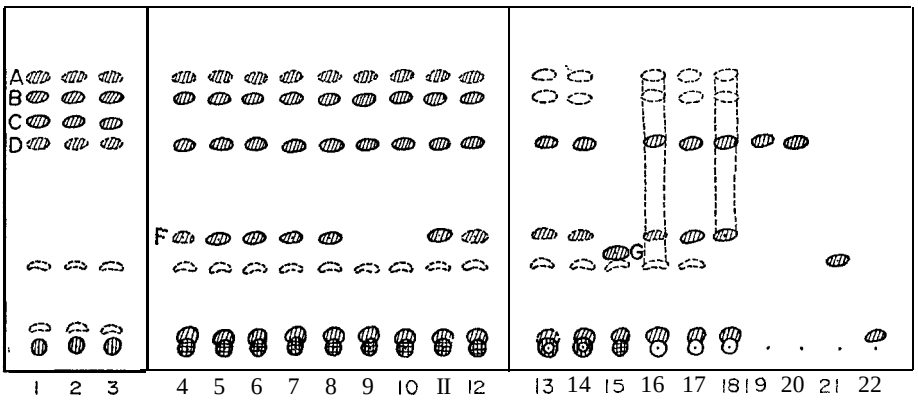

FIG. 1. CHROMATOGRAM ON $SiO_2/CaSO_4$ IN HEXANE-ETHYL ACETATE (1: 1).
(I) *Codonopsis ussuriensis* (Rupr. et Maxim.) Hemsl.; (2) C. *pilosula* (Franch.) Nannf.; (3) *C. lanceolata* (Sieb. et Zucc.) Benth. et Hook.; (4) *Adenophora divaricata* Franch. et Savat.; (5) *A. verticillata* Fisch.; (6) *A. stenanthina* (Ledeb.) Kitag.; (7) *A. triphylla* (Thunb.) A.DC. var. *kurilensis* (Nakai) Kitam.; (8) *A. remotiflora* (Sieb. et Zucc.) Miq.; (9) *A. coronopifolia* Fisch.; (10) A. *tricuspidata* (Fisch. ex Koem. et Schult.) A.DC.; (11) *A. pereskiifolia* (Fisch. ex Roem. et Schult.) G.DON; (12) A. *gmelinii* (Spreng.) Fisch.; (13) *Campanula punctata* Lam.; (14) C. *cephalotes* Nakai; (15) C. *langsdorffiana* Fisch.; (16) C. *dasyantha* M.B.; (17) *Asyneuma japonicum* (Miq.) Briq.; (18) *Astrocodon expansus* (Rud.) Fed.; (19) β-sitosterol; (20) Stigmasterine; (21) Oleanolic acid; (22) Daucosterol.
▥ blue, ▨ lilac, ▩ violet, •j yellow.

RESULTS AND DISCUSSION

Eighteen species of Far-Eastern Campanulaceae belonging to 6 genera were studied. In certain cases, it was possible to show the correlation of their morphological and chemical properties. The results are shown in Figs. l-3.

The study of the Campanulaceae by TLC with hexane-ethyl acetate (1: 1) showed that the sterol composition for each genus is relatively constant and almost unique. From Fig. 1, it is apparent that substance D, having the same R_f value as β-sitosterol, is a common substance in *Codonopsis, Adenophora, Campanula, Asyneuma* and *Astrocodon.* However,

* The chemical identification of these compounds will be described later.

[9] F. H. CARR and E. A. PRICE, *Biochem. J.* 20,498 (1926).
[10] *Dünnschicht-Chromatographie*, ein Laboratoriumshandbuch, Herausgegeben von E. Stahl, Berlin, 1962.

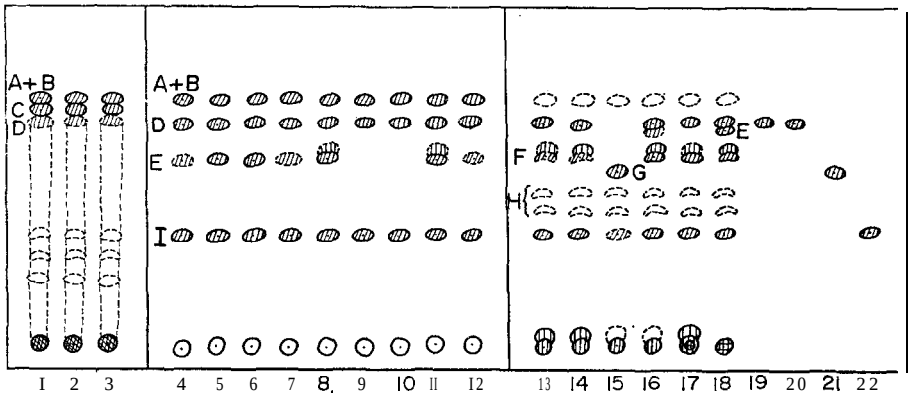

FIG. 2. CHROMATOGRAM ON $SiO_2/CaSO_4$ IN CHLOROFORM - METHANOL (10: 1). (SEE FIG. 1).

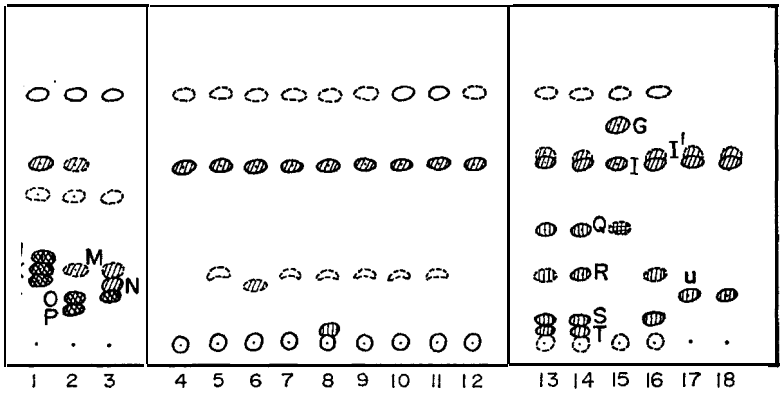

FIG. 3. CHROMATOGRAM ON $SiO_2/CaSO_4$ IN *n*-BUTANOL–ETHANOL–25% NH_4OH (7:5:2). (SEE FIG. 1).

various *Codonopsis* species contain, apart from the traces of substance D, three more compounds (A, B and C), which by their R_f values may be sterol esters or triterpenes (or 4 a-methylsterols). Substance C is specific for three *Codonopsis* species, and was not found in the other genera. All the *Adenophora* species contain identical substances (A, B and D).

The *Campanula* species studied, except for *Campanula langsdorffiana* contain mainly substance D.

The spots F to H (Fig. 1) correspond to triterpenic acids in R_f-value, as well as the nature of the reaction with the reagent; these acids are better separated in the more polar chloroform-methanol system (10: 1) (Fig. 2). Triterpenic acids are apparently characteristic components of the genera studied. The *Adenophora* species, except *A. coronopifolia* and *A. tricuspidata*, *Asyneuma japonicum, Astrocodon expansus* and the *Campanula* species, except C. *langsdorfiana,* contain an unidentified substance corresponding to spot F on the chromatogram. C. *langsdorffiana* was noted for its high content of a substance corresponding to spot G with R_f-value close to that of oleanolic acid. The essential chromatographic differences in the sterol and triterpenic acid composition for C. *langsdorfiana* corroborates

the taxonomic peculiarity of this species and justifies its separation as an independent subsection, *Heterophylla*.

It is apparent from the chromatogram in Fig. 2 that all the investigated species of the Campanulaceae, except for those of *Codonopsis*, contain substance I, which has an R_f-value identical to that of daucosterol. The absence of the substances I and D in *Codonopsis* apparently reflects the biosynthetic variation peculiar to this genus, and are in agreement with the morphological features (flower and fruit structures) and form (liana) of this group of plants.

It should be noted that platycodigenin" and polygallic acid," which are aglycones of *Platycodon grandiflorum*, were not detected in any of the genera.

The use of the more polar n-butanol-ethanol-25 % ammonia system (7: 5: 2) separated the substances which in the previous systems remained at the start (Fig. 3). These substances which are unidentified give a blue and violet colour with the Carr-Price reagent and, hence, may be terpenes or steroids.

Codonopsis species alone contain the violet reacting substances J-P. Substance 0 is probably common in C. *pilosula* and C. *lanceolata*, whereas substance P is absent in the latter. C. *ussuriensis* contains less polar substances of the same nature. Thus, the above differences in the composition of polar substances confirm the morphological peculiarity of all the three *Codonopsis* Far-Eastern species, as well as the special features of C. *pilosula*.

Some of the *Campanula* species studied likewise contain substances which with the Carr-Price reagent form blue spots (Q-T) on the chromatograms; these spots do not, however, coincide in R_f with the spots of the substances contained in the *Codonopsis* species. *Campanula punctata* and C. *cephalotes* of the *'Medium'* section are close in composition with substances Q, R, S and T, although substance R is only weakly present in the former species. Morphologically, these data justify the isolation of C. *punctata* as an independent subsection *Tulipella*.[13] *Campanula langsdorffiana* clearly differs from the above-examined species in that the substances Q-T are absent, this once again showing that its separation into the *Heterophylla* subsection was likewise correct. *Campanula dasyantha*, which morphologically belongs to the *Scapiflorae* subsection, also differs in chemical composition from C. *punctata* and C. *cephalotes* in that it probably contains only slight quantities of substances R and S.

Asyneuma japonicum and *Astrocodon expansus* both have a spot (U) of low intensity on the chromatograms. It is noteworthy that these species also contain the substance I, which was identified in all *Campanula* species. Furthermore, *Asyneuma japonicum* and *Astrocodon expansus* gave a second spot I^1 which corresponds closely to the glycoside daucosterol. The *Adenophora* species do not contain this substance: *A. remotiflora* is an exception, since it gives a blue spot with low R_f on the chromatogram. This is in agreement with the morphological, anatomical and ecological data, which show the impossibility of *A. remotiflora* and *A. tricuspidata* being in the same section.[14]

Thus, chemotaxonomic studies of Russian Far-Eastern Campanulaceae indicate that the genera differ significantly from each other, while there is a clear-cut difference within the same genus in the chemical composition of species belonging to the evolutionally well-separated sections and subsections.

[11] T. KUBOTA and H. KITANTANI, *Chem. Comm. 5,190* (1969).
[12] T. KUBOTA and H. KITATANI, *Chem. Comm.* 16, 1005 (1968).
[13] An. FEDOROV, ***Flora U.S.S.R. 24, 126 (1957).***
[14] ***G.*** I. PONOMARCHUK, ***Bull. Gr. Bot. Gard. 79, (1971).***

EXPERIMENTAL

Plant material. The plants were collected in various regions of the Soviet Far East (Maritime Province, Amur Province, Sakhalin, Khabarovsk Territory and Magadan Province) and identified by G. I. Ponomarchuk and P. G. Gorovoi. * The plant roots were collected during plant flowering and reduced to powder in air dry state.

Samples of air-dry roots, defatted with Et_2O were thoroughly extracted with hot MeOH. The MeOH extracts were then analysed by TLC (Silica gel in the solvent systems shown in Figs. l-3). Substances of triterpene and steroid nature were detected as pink spots by spraying the chromatogram with a saturated $SbCl_3$ solution in $CHCl_3$ and heating for 5 min at 105".

Duplicate plates were sprayed with conc. H_2SO_4.

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* Address: Institute of Biologically Active Substances, Siberian Department of Academy of Sciences of the U.S.S.R., Vladivostok 22, U.S.S.R. The herbarium is kept in the Institute. When identifying the species the authors consulted with Professor A. A. Fedorov who made the treatment of *Campanulaceae* family for ***Flora of the U.S.S.R.*** (1957).