[CONTRIBUTION FROM THE FOREST PRODUCTS LABORATORY, UNIVERSITY OF CALIFORNIA]

Paper Chromatography of the Tropolones of Cupressaceae II

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Heartwood of 14 species of the family *Cupressaceae* have been examined by paper partition chromatography for the tropolones present, and the results are discussed.

In the preceding paper[1] we described the paper chromatographic analysis of heartwood of several species belonging to the family *Cupressaceae* for the tropolones present. Continuing this investigation, we have analyzed additional species of the same family—in the main, those indigenous to the southwestern United States. The results are summarized in Tables I and II, together with the reports of analyses of closely related wood species taken from the literature.

The genus *Cupressus* consists of from 15 to 20 species of which the heartwood of nine has been examined for the tropolones present. The occurrence of nootkatin and of β-thujaplicin (with one exception) seems to be characteristic for the species of this genus. According to Wolf, *C. pygmaea, C. goveniana, C. abramsiana,* and *C. macrocarpa* represent a group of four related species, connected with *C. sargentii* through *C. abramsiana.*[2] The composition of their respective tropolonic fracitions clearly seems to separate the first three species of the above group from *C. macrocarpa* as well as from *C. sargentii,* paralleling to some extent the botanical characteristics.[2] Some qualitative and quantitative differences can be also noted among the three species. However, more work, particularly on the other extractives, is needed before Wolf's proposal[2] to regard all five as separate species can be substantiated.

The genus *Chamaecyparis* comprises six to seven species, of which only one (*Ch. pisifera*) has not been investigated for its tropolonic content. Of the species investigated thus far, only *Ch. nootkatensis* seems to contain nootkatin. This is rather surprising since the genus *Chamaecyparis* is botanically closely related to *Cupressus.* Should future investigations substantiate this difference, it might be of taxonomic importance for the distinction between the two genera. In this respect it might be pointed out that, of the two genera, *Chamaecyparis* is botanically closer to *Thuja,*[14] with species of the latter lacking nootkatin, as far as investigation shows.

Chamaecyparis lawsoniana has been reported to contain no tropolones.[1] However, reinvestigation of its extractives resulted in identification of β-thujaplicin, present in a rather small amount. This fact probably accounts for previous difficulties in identification.

The genus *Juniperus* comprises about 70 species, of which three have been previously examined. We investigated the heartwood of five additional species, namely, *J. monosperma, J. osteosperma, J. mexicana, J. communis,* and *J. deppeana* (Table II). *J. occidentalis* was reinvestigated. Three of the eight species examined so far seem to contain no tropolones; in species containing tropolones, nootkatin and β-thujaplicin are ordinarily present. The two species, *J. monosperma* and *J. osteosperma,* botanically difficult to differentiate, seem to show marked difference in the nature of their tropolonic fractions, the first species being much richer in tropolones, and also containing pygmaein as one of the main constituents.

(1) Eugene Zavarin and Arthur B. Anderson, *J. Org. Chem.,* **21,** 332 (1956).

(2) C. B. Wolf, El Aliso, *The New World Cypresses,* Vol. I (1948); Part I, Taxonomic and Distributional Studies of the New World Cypresses.

(3) During the progress of this work there appeared a paper by C. Enzell and H. Erdtman in *Acta Chem. Scand.,* **11,** 902 (1957), reporting the identification of nootkatin and β-thujaplicin in *Cupressus sempervirens* grown in southern France. This agrees very well with our analysis of the same species grown in California.

(4) R. E. Corbett and D. W. Wright, *Chem. and Ind.* (London), 1258 (1953).

(5) H. Erdtman in A. Todd, *Perspectives in Organic Chemistry,* Interscience Publishers, Inc., New York, 1956, p. 484.

(6) B. Carlsson, H. Erdtman, A. Frank, and W. E. Harvey, *Acta Chem. Scand.,* **6,** 690 (1952).

(7) S. Katsura, *J. Chem. Soc. Japan,* **63,** 1480 (1942).

(8) No nootkatin could be chromatographically detected in the extracts of *Chamaecyparis formosensis* of *Chamaecyparis taiwanensis* according to the private communication from Dr. Tung-bin Lo, National Taiwan University.

(9) T. Nozoe, *Bull. Chem. Soc. Japan,* **2,** 295 (1936).

(10) Y. T. Lin, K. T. Wang, and C. L. Chen, *J. Chinese Chem. Soc.,* **2,** 91 (1955).

(11) Japanese *Chamaecyparis obtusa* and Formosan *Chamaecyparis obtusa,* Sieb. and Zucc. f. *Formosana Hayata,* same as *Ch. taiwanensis,* have been regarded in this paper as two different species.

(12) T. Nozoe, *Sci. Rep. Tôhôku Univ.,* **34,** 200 (1950); *Sci. Rep. Tôhôku Univ.,* **36,** 82 (1952).

(13) T. Nakatsuka and Y. Hirose, *J. Jap. Forestry Soc.,* **37,** 196 (1955).

(14) H. L. Li, *J. of the Arnold Arboretum,* **34,** 17 (1953). The occurrence of nootkatin in *Ch. nootkatensis* could indicate the rather close relationship between this specie and the genus *Cupressus.* The known ability to hybridize with *C. macrocarpa* as well as other characteristics seems to substantiate a close relationship botanically. See "Les Cypres" by A. Camus, ed. Paul Lechevalier, Paris VI Rue de Tournon, 12, 1914, p. 24.

TABLE I[a]

Species	α-Thujaplicin	β-Thujaplicin	γ-Thujaplicin	Nootkatin	Dolabrin	Pygmaein	β-Thujaplicinol	T-11	T-10	T-4.5	T-0.1	Total	Method of Analysis
Cupressus pygmaea (Lemm.) Sarg.	—	0.1	—	0.1	0.001	0.4	—	0.4	0.3	0.1	—	1.4	Chrom.
C. sargentii, Jeps.	—	0.03	—	0.1	0.01	—	0.03	—	—	—	—	0.17	Chrom.
C. abramsiana, C. B. Wolf	—	0.04	—	0.02	0.001	—	—	0.01[b]	—[b]	—	—	0.07	Chrom.
C. goveniana, Gord.	—	0.008	—	0.0008	0.0004	0.005	—	0.05[b]	—[b]	—	—	0.02	Chrom.
C. arizonica, Greene	—	0.0003	0.002	0.008	—	—	0.0003	—	—	—	—	0.01	Chrom.
C. sempervirens, L.[3]	—	0.2	—	2.0	—	—	—	—	—	—	—	2.2	Chrom. & Prep.
C. macrocarpa, Hartw.[1,4,c]	—	+	—	0.2	—	—	—	—	—	—	—	0.2	Chrom. & Prep.
C. torulosa[5,c]	—	—	—	+	—	—	—	—	—	—	—	Unkn.	Unkn.
C. macnabiana[5,c]	+	+[b]	+[5]	+	—	—	—	—	—	—	—	Unkn.	Unkn.
Chamaecyparis lawsoniana[9] (A. Murr) Parl	—	0.0002	—	—	—	—	—	—	—	—	—	0.0002	Chrom.
Ch. nootkatensis,[c] (D. Don) Spach[1,6]	—	—	—	0.1	—	—	—	—	—	—	—	0.1	Chrom. & Prep.
Ch. tyoides,[c] (B.S.P.)	+	+	+	—	—	—	—	—	—	—	—	Unkn.	Chrom.
Ch. formosensis,[c] Matsum.[7-9]	—	+	—	—	—	—	—	—	—	—	—	Unkn.	Chrom. & Prep.
Ch. taiwanensis,[c] Masam. et Suz.[8,10-12]	+	+	—	—	—	—	—	—	—	—	—	Unkn.	Chrom. & Prep.
Ch. obtusa,[c] Sieb. et Zucc.[11,12]	—	—	—	—	—	—	—	—	—	—	—	—	Prep.

[a] Numbers denote the approximate amount of a tropolone in %, based on dry weight of wood. [b] In this case it has not been determined which of the two compounds is present, or whether both are. [c] These analyses have been taken from the literature.

TABLE II[a]

Species	α-Thujaplicin	β-Thujaplicin	γ-Thujaplicin	Nootkatin	Dolabrin	Pygmaein	β-Thujaplicinol	T-11	T-10	T-4.5	T-0.1	Total	Method of Analysis
Papuacedrus torricellensis, Li	—	0.07	—	—	—	—	—	—	—	—	0.05	0.12	Chrom.
Juniperus occidentalis,[1] Hook	—	—	—	—	—	—	—	—	—	—	—	—	Chrom.
J. communis, L.	—	0.001	—	0.003	—	0.0003	—	—	—	0.0006	—	0.005	Chrom.
J. monosperma, (Englm) Sarg	—	0.0002	—	0.008	0.0001	0.008	—	—	—	—	—	0.16	Chrom.
J. osteosperma, (Torr) Little	—	0.0001	—	Trace	—	—	—	—	—	—	—	0.0001	Chrom.
J. deppeana, Steud.	—	0.003	—	0.04	0.0002	—	—	—	—	—	—	0.043	Chrom.
J. mexicana, Spreng.	—	—	—	—	—	—	—	—	—	—	—	—	Chrom.
J. virginiana,[1] L.[b]	—	—	—	—	—	—	—	—	—	—	—	—	Chrom.
J. chinensis,[13] L.[b]	—	0.0004	—	0.008	—	—	—	—	—	—	—	0.0084	Prep.

[a] Numbers denote the approximate amount of tropolone in %, based on dry weight of wood. [b] These analyses have been taken from the literature.

Examination of the heartwood of *Papuacedrus torricellensis* (formerly *Libocedrus torricellensis*)[14] resulted in identification of β-thujaplicin. A rather intensive spot at a lower R_f, exhibiting the characteristic reactions of tropolones, was also present (Table III).

Several new tropolones have been encountered in this investigation. Of these, one, designated pygmaein, has been isolated preparatively. It represents a methoxy thujaplicin, the structure of which will be the subject of a forthcoming paper. A mixture of two hydroxythujaplicins, tentatively designated T-11 and T-10,[15] has been isolated from the same source, *Cupressus pygmaea*. Two further characteristic compounds, one designated T-4.5 occurring in *C. pygmaea* and *J. communis* extracts, the other designated T-0.1, present in the extract of *Papuacedrus torricellensis*, are also likely tropolonic in nature.

The phosphoric acid chromatographic procedure was used throughout most of the work.[1] The recently developed method of Wachtmeister and Wickberg[16] has been found superior for the separation of nootkatin in the presence of rather large amounts of the hydroxytropolones, T-11 and T-10, and for the separation of the latter two from each other. With this method there are larger differences in R_f values, and nootkatin shows less tendency to streak. In both procedures, the spots seem to appear in the same order, with exception of β-dolabrin and the three hydroxy tropolones, β-thujaplicinol, T-11, and T-10, which are decidedly retarded in the second method.

It seemed advantageous to express the ability of individual tropolones to migrate on paper in terms of R_β values, defined as the ratio of the distance migrated by a certain tropolone to the distance migrated by β-thujaplicin. The R_β values were found to be much more constant than R_f values when the same procedure was used. β-Thujaplicin was used as a reference since it appears to be the most common tropolone in *Cupressaceae*. The R_β values of the most important tropolones are tabulated in Table III for both the phosphoric acid and the Wachtmeister and Wickberg procedures.

TABLE III

Tropolone	R_β (21.2% H_3PO_4·Iso-octane)	R_β (Wachtmeister and Wickberg-Iso-octane)	Color (Phosphoric Acid–$FeCl_3$)
α-Thujaplicin	8.6	1.3	Grey-green
β-Thujaplicin	1.0	1.0	Brown
γ-Thujaplicin	0.50	0.88	Green-brown
Nootkatin	9.2	1.38	Green-buff
Dolabrin	1.6	0.72	Tan
Pygmaein	3.8	1.2	Green-g4ey
β-Thujaplicinol	1.64	0.75	Purple
T-11	11.0	1.07	Purple
T-10	10.0	0.76	Purple
T-4.5	4.5	1.2	Green-grrey
T-0.1	0.1	0.61	Blue-green

(15) The numbers refer to the R_β values in Table III.

(16) C. A. Wachtmeister and B. Wickberg, *Acta Chem. Scand.*, **12**, 1335 (1958).

EXPERIMENTAL

Origin of samples. The samples of *Cupressus arizonica*, *Juniperus osteosperma*, *J. monosperma*, and *J. deppeana* wood came from Arizona; the sample of *J. mexicana* from Texas, and the sample of *Papauacedrus torricelensis* from New Guinea. More detailed designation of locations is unavailable. The sample of *Juniperus communis* wood was taken from a planted specimen at the Institute of Forest Genetics, Placerville, Calif., original source unknown. The sample of *Cupressus pygmaea* was secured in the Pygmy Forest, east of Fort Bragg, Calif.; the sample of *C. goveniana* on Huckleberry Hill near Carmel, Calif.; the sample of *C. sargentii* on Cedar Mountain, Alameda County, Calif., and the sample of *C. abramsiana* was obtained near Bonny Doon in the Santa Cruz mountains, Calif.[2] The wood sample of *Cupressus sempervirens* was obtained from a planted tree growing on the grounds of this Laboratory, original source unknown. The sample of *Chamaecyparis lawsoniana* wood was collected near Highway 199, about 50 moles from the Pacific Coast, in California, and the wood of *Juniperus occidentalis* was obtained from the Black Mountain forest, near Susanville, Calif. When necessary, the identification of wood samples was made in cooperation with the University of California Herbarium.

Isolation of the tropolone fraction. The heartwood portion was separated from the rest of a sample on the basis of color and other characteristics, ground to 20 mesh in a Wiley mill, and extracted with acetone for 8 hr. The sample size was from 50 to 2000 g., depending on the quantity of wood available. The acetone was removed from the extract by distillation, and the residue was steam distilled until only negligible amounts of organic matter volatilized, giving practically no green color with copper acetate in chloroform solution. The distillate was next exhaustively extracted with chloroform in the presence of an excess of copper acetate, and the extract was shaken with an excess of 10% hydrochloric acid to decompose the formed tropolone chelates. In some cases it was found advantageous to treat the evaporation residue of the acetone extract with 3 times its volume of chloroform prior to steam distillation, to precipitate the bulk of phlobaphenes that usually interfere by occluding the volatile material. In some cases the steam distillation of the chloroform-soluble evaporation residue was substituted by exhaustive extraction with *n*-hexane; this did not offer any particular advantage over the steam-distillation method.

The extracts obtained by these methods were shaken with an excess of 10% sodium hydroxide in 3 portions, the aqueous phase was neutralized with 10% hydrochloric acid, treated with an excess of ammoniacal 5% copper acetate solution, and exhaustively extracted with chloroform. The chloroform extracts were dried with sodium sulfate and filtered. The filtrate was concentrated by distillation to a volume of 5 to 25 ml., treated with hydrogen sulfide to decompose the copper chelates,[17] and filtered. The evaporation residues of the filtrate were sublimed at 0.5–1.0 mm. and 200°, to remove tarry impurities which seem to cause an excessive streaking on chromatograms.

(17) It has been observed that hydroxy tropolones T-11 and T-10 slowly decomposed if kept in the form of their copper complexes, giving no reaction with ferric chloride. For this reason decomposition of the copper complexes with hydrogen sulfide was conducted as soon as possible.

In all of the above separation procedures, each step was tested for completeness by placing a small portion of the material in question on the phosphoric acid–impregnated paper and treating the spot with ferric chloride. When the mixture being tested was so strongly colored as to mask the color of iron complexes, a small portion of iso-octane was allowed to migrate through the spot, carrying with it any tropolone present and leaving behind the interfering substances.

Chromatography. The phosphoric acid procedure of Zavarin and Anderson[1] was used throughout most of the work. Some streaking tendency of the tropolones was still encountered, particularly in the case of nootkatin. Removal of the highly colored, phlobaphenic impurities from the tropolonic fractions seemed to be of utmost importance for control of streaking. Development of the chromatograms at a constant temperature near 21° was also helpful. The streaking was reduced when Whatman No. 3 was substituted for Whatman No. 1 paper.

As in the phosphoric acid procedure, the R_f values of nootkatin and hydroxytropolones T-11 and T-10 are rather close. The resolution was achieved by the dimethylsulfoxide procedure of Wachmeister and Wickberg,[16] using iso-octane as the developing solvent. This method completely separated the three compounds; in addition, nootkatin showed much less tendency to streak.

The 5% ferric chloride solution was mostly used as chromogenic agent. The tendency of the spots obtained to fade with time was overcome by neutralizing the phosphoric acid of the paper with gaseous ammonia. This treatment also regenerated the partially faded spots. The color of the spots was changed characteristically by this treatment.

Another chromagenic agent used was a solution of diazotized *o*-aminobiphenyl, prepared by dissolving 0.1 g. of *o*-aminobiphenyl in 0.3 ml. of 10% hydrochloric acid, treating the resulting mixture with 1 ml. of 10% sodium nitrite for 5 min. with stirring, decomposing the excess of nitrous acid with 0.5 g. of urea, and diluting the whole to 10 ml. The solution gave a characteristic purple color with γ-thujaplicin, and buff to pink colors with the other tropolones after the sprayed paper was treated with gaseous ammonia. The diazo compounds of the other amines tested, such as aniline, benzidine, *p*-nitroaniline, *p*-aminobenzoic acid, and sulfanilic acid, were much less stable to the treatment with ammonia, and produced background color which interfered with that of the formed azodyes.

For semiquantitative evaluation of the concentration of tropolones in wood, the intensities of the spots containing known amounts of substance were compared visually with the intensity of the spots from the extracts. Greater accuracy was considered unnecessary because of the natural variation of the tropolone content within the species.

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Friedel-Crafts Reactions of Some Acylals

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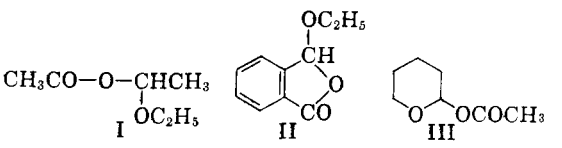
1-Ethoxyethyl acetate (I) reacts with anisole to form *p*-methoxyacetophenone and 4,4′-vinylidenedianisole (IV) if sufficient aluminum chloride is present, or 4,4′-ethylidenedianisole with less aluminum chloride. The IV was formed also starting with *p*-(1-ethoxyethyl)anisole, anisole and aluminum chloride. The products from I, toluene and aluminum chloride were *p*-methylacetophenone, 1,1-di-*p*-tolylethane and some meta isomer of the latter. 3-Ethoxyphthalide, toluene and aluminum chloride yielded 3-*p*-tolylphthalide and an unidentified carboxylic acid. The reaction of tetrahydropyran-2-yl acetate, toluene, and aluminum chloride gave rise only to an intractable sirup.

A few acylals have been subjected to reactions under Friedel-Crafts conditions but these have all been of carbohydrate nature. Such a reaction is the conversion of glucose pentaacetate into tetraacetylglucosyl chloride[1] by means of aluminum chloride. Another reaction is the synthesis of aryl tetraacetylglucosides by fusing glucose pentaacetate with a phenol[2] in the presence of aluminum chloride (or zinc chloride). Finally, there is the glucosylation of benzene and other aromatic hydrocarbons by reaction with glucose pentaacetate or tetraacetylglucosyl chloride and aluminum chloride[3] or hydrogen fluoride.[4]

The purpose of the present investigation was to subject simpler acylals to Friedel-Crafts conditions, and for this purpose 1-ethoxyethyl acetate (I), 3-ethoxyphthalide (II), and tetrahydropyran-2-



(1) A. Kunz and C. S. Hudson, *J. Am. Chem. Soc.*, **48**, 1978 (1926).

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