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D-1-*O*-METHYL-*MUCO*INOSITOL IN HIGHER PLANTS

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Abstract— D-1-*O*-Methyl-*muco*-inositol was isolated from the needles of *Juniperus communis* The occurrence of this compound in many gymnosperms and in a few angiosperms was demonstrated

INTRODUCTION

O-METHYLINOSITOLS are widespread in higher plants; however, D-1-*O*-methyl-*muco*inositol so far has been found only in the wood of *Phyllocladus trichomanoides*.[1] Since the optical rotation of the isolated compound was not determined, it is not known which of the two possible optical antipodes occurs in nature. Recently a methylated *muco*inositol was isolated by Utkin[2] from the berries of *Juniperus foetidissima*, but in this case the position of the methyl group was not determined.

The present paper reports the isolation and identification of D-1-*O*-methyl-*muco*inositol from the needles of *Juniperus communis* In addition it is shown that D-1-*O*-methyl-*muco*-inositol occurs in some angiosperms and in practically all conifers except members of the Pinaceae

RESULTS AND DISCUSSION

A hitherto unidentified spot was noticed, when two dimensional paper chromatograms of leaf extracts of various gymnosperms were sprayed with alkaline silver nitrate Small amounts of this substance were isolated subsequently by large scale paper chromatography Several tests, such as electrophoresis in various buffers, hydrolysis, demethylation and periodate oxidation, indicated that the unknown compound was an *O*-methyl derivative of a cyclitol not identical with any of the commonly known *O*-methylinositols

Identification

The compound was isolated by column chromatography on a larger scale and C and H analysis was in good agreement with the theoretical values of an *O*-methylinositol. Treatment of the substance with boiling hydriodic acid gave an easily crystallizable product, which was identified as *muco*inositol by means of paper chromatography in solvents (a), (b) and (d) (see Experimental), as well as by electrophoresis in borate buffer In addition, the melting point of hexacetate of the demethylated compound (177–179°) agreed well with that (177–178°) reported for the melting point of *muco*inositol-hexaacetate [3] Since there are two optical antipodes and one optical inactive form of an *O*-methyl-*muco*inositol the specific rotation of the isolated compound was determined Its $[\alpha]_D^{22}$ −53·2° (*c* 1 5, ethanol) is very close to the value found by Angyal *et al* [4] for D-1-*O*-methyl-*muco*inositol

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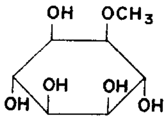
[1] S K ADHIKARI, R A BELL and W E HARVEY, *J Chem Soc* 2829 (1962)

[2] L M UTKIN, *Khim Prir Soedin* 4, 277 (1968)

[3] S J ANGYAL and L ANDERSON, *Adv Carbohyd Chem* **14**, 135 (1959)

[4] S J ANGYAL, V J BENDER, P T GILHAM, R M HOSKINSON and M. E. PITMAN, *Austral J Chem* **20**, 2109 (1967)

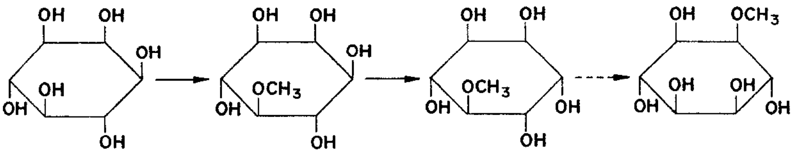
synthesized from L-quebrachitol The pentabenzoate had melting point identical with that described for D-1-*O*-methyl-*muco*inositol pentabenzoate (97–103°) [4] The substance also co-chromatographed in solvents (a), (b) and (d) with authentic D-1-*O*-methyl-*muco*inositol, which was a generous gift from Professor Angyal In addition, co-electrophoresis in borate buffer gave identical R_f values These data demonstrate the identity of the compound isolated from the needles of *Juniperus communis* with D-1-*O*-methyl-*muco*inositol (Fig 1)



D-1-*O*-methyl-*muco*inositol

Distribution

The hot water extracts corresponding to 2 mg dry weight of the needles or leaves of the species listed in Tables 1 and 3 were chromatographed two dimensionally in solvent system a and b, and system b and c Since the minimal amount of D-1-*O*-methyl-*muco*inositol detected by the silver nitrate reagent was 0 005 mg, a negative result in Table 1 means that the cyclitol is present in a concentration less than 0 25 per cent of dry weight In the positive cases, the amount was generally 10–30 times higher than the detectable minimal concentration as shown in Table 2 Some negative cases were investigated by more sensitive methods including labelling via photosynthesis[5] in $^{14}CO_2$, but nevertheless no D-1-*O*-methyl-*muco*inositol was found To evaluate the systematic relevance of the distribution of the D-1-*O*-methyl-*muco*inositol within the plant kingdom it is necessary to consider the biogenetic relationship of *O*-methyl-inositols Though in most cases the methyl derivatives are synthesized by methylation of corresponding inositols, it has been shown[6] that D-pinitol is synthesized via epimerization of sequoyitol (Fig 2)



myo-inositol sequoyitol D-pinitol D-1-*O*-methyl-*muco*inositol

The formation of D-1-*O*-methyl-*muco*inositol is very unlikely to proceed by methylation of *muco*inositol since our investigation never revealed the presence of the latter However the labelling sequence *myo*inositol, sequoyitol, D-pinitol, D-1-*O*-methyl-*muco*inositol after photosynthesis in $^{14}CO_2$ indicates strongly that the latter is formed by epimerization of D-pinitol as suggested in Fig 2 (P Dittrich and O Kandler, in preparation) According to this scheme D-1-*O*-methyl-*muco*inositol is the last member of the 'sequoyitol family' of inositol methyl ethers

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[5] O Kandler, *Ber Dt Bot Ges* 77, 62 (1964)

[6] H Kindl and O Hoffmann-Ostenhof, *Fortschr Chem Org Nat* **24**, 149 (1966)

Table 1 Gymnosperms investigated for presence of *O*-Methylinositols

Class and family	Species		Cyclitol	
		Sequoyitol	D-Pinitol	D-1-*O*-Methyl-*muco*inositol
Cycadopsida				
Cycadaceae	*Cycas revoluta*	+	+	—
Zamiaceae	**Dioon edule*	+	—	—
	D spinolosum	+	—	—
	Encephalartos hildebrandtii	+	+	—
	**Zamia lindenii*	+	—	—
Ginkgoaceae	*Ginkgo biloba*	+	+	—
Coniferopsida				
Araucariaceae	*Agathis robusta*	+	—	+
	Araucaria excelsa	+	—	+
Cephalotaxaceae	**Cephalotaxus fortunei*	+	+	+
	C harringtonia	+	+	+
Cupressaceae	*Actinostrobus pyramidalis*	—	+	+
	**Callitris balansae*	—	+	+
	C canescens	—	+	+
	**C quadrivalvis*	—	+	+
	Chamaecyparis pisifera	+	+	+
	Cupressus sempervirens	+	+	+
	**Fitzroya patagonica*	—	+	—
	Juniperus communis	+	+	+
	**Lidocedrus decurrens*	—	+	+
	**L tetragona*	+	+	+
	Thuja occidentalis	—	+	+
	Thujopsis dolobrata	+	+	+
	Widdringtonia cupressoides	—	+	+
Pinaceae	*Abies alba*	+	+	—
	Cedrus libani	—	+	—
	**Keteeleria davidiana*	+	+	—
	Larix decidua	+	+	—
	Picea abies	+	+	—
	Pinus nigra	—	+	—
	P silvestris	—	+	—
	Pseudotsuga taxifolia	+	+	—
	Tsuga canadensis	+	+	—
Podocarpaceae	**Dacrydium araucarioides*	—	—	+
	**D cupressinum*	—	—	+
	Mikrocachrys tetragona	+	+	+
	**Pherosphaera fitzgeraldi*	—	—	+
	**Phyllocladus trichomanoides*	+	+	+
	Podocarpus macrophyllus	+	+	+
	**Saxegothaea conspicua*	+	—	+
Taxodiaceae	**Arthrotaxis selaginoides*	+	+	—
	Cryptomeria japonica	+	+	+
	Cunninghamia sinensis	+	+	—
	Metasequoia glyptostroboides	+	+	+
	Sciadopitys verticillata	+	+	+
	Sequoia sempervirens	+	+	+
	Sequoiadendron giganteum	+	+	+
	**Taiwania cryptomerioides*	—	—	+
	**Taxodium mucronatum*	+	+	+

Table 1 —*Continued*

Class and family	Species	Cyclitol Sequoyitol	 D-Pinitol	 D-1-*O*-Methyl-*muco*inositol
Taxopsidae				
	Torreya californica	+	+	+
Taxaceae	*T nucifera*	+	—	+
	Taxus baccata	+	+	+
Chlamydospermae				
Ephedraceae	*Ephedra gerardiana*	—	—	—
Gnetaceae	*Gnetum gnemon*	—	—	—
Welwitschiaceae	*Welwitschia mirabilis*	—	—	+

* Herbarium specimen

The distribution pattern of the various members of this family within the plant kingdom can be considered a consequence of the acquisition or loss of the epimerases or the methylating enzyme during evolution As seen in Table 1 sequoyitol and D-pinitol are present in all classes of gymnosperms except the Chlamydospermae, while D-1-*O*-methyl-*muco*inositol is restricted to the Coniferopsida and Taxopsida The Pinaceae is the only family within the Coniferopsida lacking the D-1-*O*-methyl-*muco*inositol The inability to epimerize pinitol is a second chemotaxonomic criterion beside the lack of biflavonyls[7] which separates the Pinaceae from all other conifers

It is interesting to notice that the Chlamydospermae are divided into two groups in respect to the occurrence of *O*-methylinositols While Ephedraceae and Gnetaceae do not contain any *O*-methylinositols, *Welwitschia* contains detectable amounts of 1-*O*-methyl-*muco*inositol Although sequoyitol and D-pinitol were not found, it is possible that they are present as in all other plants with 1-*O*-methyl-*muco*inositol, however in minimal concentration The occurrence of an inositol methyl ether in *Welwitschia* may indicate a closer relationship of this unique plant to the Coniferopsida and a more distinct separation from the

Table 2 D-1-*O*-Methyl-*muco*inositol content in needles of various gymnosperms

Species	Content (mg/g dry wt)
Araucaria excelsa	74 0
Callitris macleayana	33 1
Cephalotaxus harringtonia	41 2
Cupressus sempervirens	45 2
Juniperus communis	56 8
Podocarpus macrophyllus	32 6
Sequoiadendron giganteum	52 5
Sciadopitys verticillata	56 1
Taxus baccata	71 4

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[7] R. Hegnauer, *Chemotax d Pflanzen*, Vol 1, Birkhauser, Verlag (1962)

TABLE 3 ANGIOSPERMS (SPECIES CONTAINING D-PINITOL) INVESTIGATED FOR PRESENCE OF D-1-*O*-METHYL *muco*INOSITOL

Family	Species	D-1-*O*-Methyl-*muco*inositol
Aristolochiaceae	*Aristolochia clematis*	—
Asclepiadaceae	*Hoya carnosa*	—
Caryophyllaceae	*Dianthus caryophyllus*	—
	Lychnis flos-cuculi	—
Cistaceae	*Cistus laurifolius*	+
	C salvifolius	+
	Helianthemum carnaecystus	+
Euphorbiaceae	*Euphorbia macrostegia*	—
Nyctaginaceae	*Bougainvillea glabra*	—
Papilionaceae	*Trifolium incarnatum*	—
Tamaricaceae	*Tamarix hispida*	—

two other families. Some morphological criteria like the formation of cones are in agreement with such an assumption

While the formation of *O*-methylinositols within the gymnosperms is restricted to the synthesis of the 'sequoyitol family', the angiosperms show a considerable variation.[6] According to Fig. 2 it is also likely that only those angiosperms synthesizing D-pinitol could form D-1-*O*-methyl-*muco*inositol Therefore, some members of such families were investigated As shown in Table 3, only members of the Cistaceae were found to contain D-1-*O*-methyl-*muco*inositol Although the number of angiosperms investigated is very limited, these findings suggest that the epimerization of D-pinitol is very rare among the angiosperms while it is very common within the gymnosperms.

EXPERIMENTAL

Materials Plant material for analysis was obtained from the Botanical Garden or from the Bavarian State Herbarium Munchen

Methods If not otherwise stated the samples of needles were assayed for cyclitols by two dimensional paper chromatography (Whatman No 1, descending), using the following solvents (v/v) (a) *n*-butanol–pyridine–acetic acid–water (60 40 3 30), (b) *n*–butanol–propionic acid–water (75 35 50), (c) 88% phenol–water–acetic acid–1 M EDTA (84 16 1·0 1), (d) acetone–water (85 15)

High voltage paper electrophoresis was performed on Whatman No 3 paper with 0 1 M sodium molybdate buffer pH 5 0 or 0 05 M sodium tetraborate, according to Weigel [8]

Column chromatography on cellulose powder,[9] on charcoal-celite[10] or on Dowex ion-exchange resin (borate form)[11] was carried out as described Inositols and carbohydrates were detected by spraying the paper chromatograms with alkaline silver nitrate [12] M ps were determined with the Kofler-apparatus

The optical rotation was read with a 0 01° Zeiss Kreispolarimeter Demethylation and preparation of derivatives such acetates and benzoates were performed according to well-known procedures Quantitative determination of D-1-*O*-methyl-*muco*inositol was carried out by periodate oxidation as described in the literature [13,14]

Isolation Needles of *Juniperus communis* were dried at 100°, ground and extracted with hot H_2O

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[8] H WEIGEL, *Adv Carbohyd Chem* **18,** 61 (1962)

[9] S J ANGYAL, P T GILHAM and C G MACDONALD, *J Chem Soc* 1417 (1957).

[10] R L WHISTLER and D F DURSO, *J. Am Chem Soc* **72,** 677 (1950)

[11] G R NOGGLE and L P ZILL, *Arch Biochem Biophys* **41,** 21 (1952)

[12] W E TREVELYAN, D P PROCTER and J S HARRISON, *Nature* **166,** 444 (1950)

[13] G AVIGAD, *Carbohyd Res* **11,** 119 (1969)

[14] S. R SARFATI and SZABÒ, *Carbohyd Res* **11,** 571 (1969)

Polysaccharides, pectins and other polymers were removed from the extract by acetone and MeOH precipitation After fermentation with yeast the extract was chromatographed on a charcoal-celite column (elution with H_2O) The effluent contained no other carbohydrate than *myo*-inositol, sequoyitol, pinitol and the unknown cyclitol *Myo*-inositol and sequoyitol were removed by further chromatography on a cellulose column (acetone-H_2O 85 15) To separate the unknown compound from pinitol, the solution was chromatographed on a Dowex 1 × 8 (200–400 mesh) borate form column When eluted with 0 001 M $Na_2B_4O_7$, fractions 1–6 contained only the unknown compound, fractions 13–30 contained pure pinitol and the fractions 7–12 contained a mixture of both substances After filtration over Dowex cation-exchange resin and removal of the H_3BO_3 by multiple evaporations with MeOH, the unknown substance was obtained as a syrup Since all attempts to crystallize the compound failed, it was dried with Et_2O (Found C, 44 2, H, 7 36, $C_7H_{14}O_6$ requires C, 43 3, H, 7 21%)

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Key Word Index—*Juniperus communis*, Cupressaceae, Gymnospermae, Angiospermae, cyclitols, D-1-*O*-methyl-*muco*inositol, chemotaxonomy