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THE OCCURRENCE OF IMBRICATOLIC ACID IN *CUPRESSUS* RESINS

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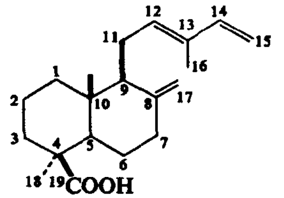
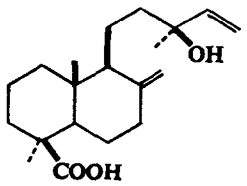
Abstract—Imbricatolic acid (a dihydroisocupressic acid) has been isolated from the resin of *Cupressus nevadensis* Abrams and shown to be present in the resins of all the remaining American and Asian *Cupressus* species examined but absent from that of the single European species *C. sempervirens* L.

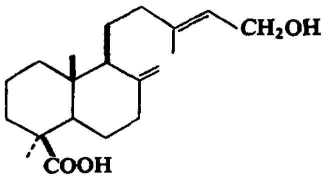
THE CHEMISTRY of the genus *Cupressus* (Cupressaceae) has received some attention[1,2] but the only exuded resin which has been examined is that of *C. sempervirens* L.[3] This was found to contain *trans*-communic acid (I), cupressic acid (III), and isocupressic acid (IV),[3a] as well as a number of related compounds and diterpene phenols.[3b,c] In a gas chromatographic survey of the compositions of most of the remaining members of the genus we observed the presence of a compound with a high retention value absent from *C. sempervirens* resin. This appeared to be a hydroxy acid (change of retention time on acetylation) but did not correspond to any reference compound available to us and was therefore isolated.



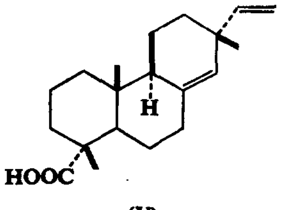
(I) 12,13-*trans*
(II) 12,13-*cis*



(III)



(IV)



(V)

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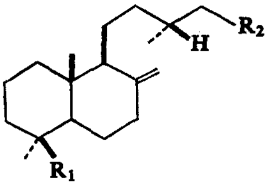
[1] H. ERDTMAN and T. NORIN, *Fortschr. der chemie Organischer Naturstoffe* **24**, 206 (1966).

[2] E. ZAVARIN, L. V. SMITH and J. G. BICHO, *Phytochem.* **6**, 1387 (1967).

[3] *a.* L. MANGONI and M. BELARDINI, *Gazz. chim. Ital.* **94**, 1108 (1964); *b.* L. MANGONI and M. BELARDINI, *Gazz. chim. Ital.* **96**, 206 (1966); *c.* L. MANGONI and R. CAPUTO, *Gazz. chim. Ital.* **97**, 908 (1967).

We had resin of *C. nevadensis* Abrams in largest amount and this was used as source. GLC of this resin (after methylation) showed the unknown compound as well as sandaracopimaric acid (V) and *trans*- and *cis*-communic acid (I and II).* Most of the latter was polymerized during the work-up of the resin, and chromatography of the acid fraction on silica gel yielded sandaracopimaric acid (3 per cent) and the almost pure hydroxy acid (9·5 per cent) which was further purified by chromatography of its methyl ester. Although homogeneous by the usual criteria, neither the acid nor its methyl ester could be crystallized. Spectral data suggested a close relationship to isocupressic acid but the apparent absence of the side-chain (13, 14) double bond. This dihydro derivative of isocupressic acid (imbricatolic acid) has been isolated from an *Araucaria* resin[4] and comparison of its constants with those of our material indicated their probable identity. $LiAlH_4$ reduction of the methyl ester gave, in good yield, a crystalline diol which was shown by direct comparison to be identical with imbricatadiol (VII), which had previously been prepared[4d] from methyl imbricatolate as well as isolated direct from the *Araucaria* resin. A direct comparison of the acid methyl esters was not made, but the possibility that our compound is in fact the unknown 13,14-dihydroagatholic acid (VIII) may be discounted by the change in chemical shift of the C-18 protons which occurs when the ester is reduced to the diol and by other spectroscopic data.



(VI) $R_1 = COOH, R_2 = CH_2OH$
(VII) $R_1 = R_2 = CH_2OH$
(VIII) $R_1 = CH_2OH, R_2 = COOH$

The occurrence of imbricatolic acid in the resins of *Cupressus* species† is summarized in Table 1. It is not possible to derive percentage compositions of the resins directly from GLC results because of the variable degree of polymerization of the communic acids, and proportions are therefore given relative to sandaracopimaric acid. Imbricatolic acid has also been observed in the resins of *Juniperus* (sections *Oxycedrus* and *Caryocedrus*) and in the hybrid *Cupressocyparis* species. It is only very exceptionally present in *Juniperus* section *Sabina*, and it appears to be absent from all other genera of the family Cupressaceae. More detailed studies of these resins will be reported later.

EXPERIMENTAL

Gas chromatography was carried out with a Pye "Panchromatograph" using glass columns and a flame ionization detector. Three stationary phases were used; 10% PEGA (5 ft.) at 205°, 3% Apiezon L (9 ft) at 225°, and 3% XE-60 (5 ft) programmed 180–210° at 2°/min. Resin samples were run after methylation with diazomethane, and also after subsequent acetylation with pyridine–acetic anhydride. Methyl *O*-acetylimbricatolate was stable on all three columns but methyl imbricatolate itself was largely absorbed by the PEGA and also suffered considerable decomposition on the APL column (reduced peak size and tailing). NMR spectra were determined in $CDCl_3$ solutions at 60 MHz.

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* Both *trans*- and *cis*-communic acids and sandaracopimaric acid have been detected in all *Cupressus* resins examined, including that of *C. sempervirens* (cf. Ref. 3).

† We follow the botanical classification of Dallimore and Jackson (revised by S. G. Harrison) in *A Handbook of Coniferae and Ginkgoaceae*, 4th ed., Edward Arnold, London (1966).

[4] *a.* G. Weissmann and K. Bruns, *Naturwiss.* **52**, 185 (1965); *b.* G. Weissmann, K. Bruns and H. Fr. Grutzmacher, *Tetrahedron Letters* 4623 (1965); *c.* K. Bruns and G. Weissmann, *Tetrahedron Letters* 1901 (1966); *d.* K. Bruns, *Tetrahedron* **24**, 3417 (1968).

Table 1. The distribution of imbricatolic acid in *Cupressus* species and related hybrids

Species	Source and number of samples*	Proportion of imbricatolic acid†
Cupressus sempervirens L.	cultivated 4	0
C. cashmeriana Royle ex Carr.	cultivated 4	23–89
C. torulosa Don	cultivated 6	12–210‡
C. duclouxiana Hickel	cultivated 2	41–360
C. funebris Endl.	cultivated 4	275–660
C. macrocarpa Hartweg	wild 2	88–800
	cultivated 1	165
C. guadalupensis S. Watson	cultivated 3	66–130
C. forbesii Jepson	wild 1	570
	cultivated 2	105–560
C. goveniana Gordon	wild 2	280–305
C. pygmaea (Lemm.) Sargent	wild 1	280
	cultivated 2	89–630
C. abramsiana C. B. Wolf	wild 3	130–320
C. sargentii Jepson	wild 4	120–460
	cultivated 1	540
C. arizonica Greene	wild 2	140–165‡
	cultivated 1	330
C. glabra Sudworth	wild 1	275
	cultivated 2	195–700
C. nevadensis Abrams	wild 3	160–600
	cultivated 1	1640
C. stephensonii C. B. Wolf	wild 1	360
C. lusitanica Miller	cultivated 2	62–67
C. bakeri Jepson§	wild 3	135–140
C. macnabiana Murray	wild 5	9–150
C. lusitanica x *macrocarpa*‖	cultivated 1	45
× *Cupressocyparis leylandii* (Dall. and Jacks.) Dallimore	cultivated 1	180
sp. *nova* (*Cupressus glabra* x *Chamaecyparis nootkatensis*)	cultivated 1	36

* Collected from cultivated trees grown in England, Kenya, or California; wild specimens from various groves listed by C. B. Wolf (*El Aliso* **1**, 1 (1948)) or J. R. Griffin and C. O. Stone (*Madroño* **19**, 19 (1967)).

† Area of GLC peak relative to that of sandaracopimaric acid = 100.

‡ In some samples up to 55% of the imbricatolic acid is present as the *O*-acetyl derivative (cf. Ref. 4*b*). Small amounts (<5%) of *O*-acetyl imbricatolic acid may be present in other species.

§ Both subspecies examined.

‖ Reported by W. G. Dyson and G. A. Herbin, *Phytochem.* **7**, 1339 (1968).

Isolation of Resin Acids

Resin was collected from *Cupressus nevadensis* Abrams growing in the Piute Mountains near Bodfish, Kern Co., California. The coarsely powdered resin (39 g) was digested with ether (400 ml) at room temp. overnight. The ether solution was decanted and the extraction repeated. Residual polymer ("polycommunic acid"), after drying, weighed 7·5 g. The combined ether extracts were divided into acid and neutral material by extraction with aq. sodium carbonate solution. Considerable further polymerization of communic acid occurred during evaporation of the acid fraction (21 g) and 9·5 g was insoluble in CH_2Cl_2 in which the soluble portion was chromatographed on silica gel (400 g). Elution with CH_2Cl_2 gave crystals (1·2 g) which, crystallized from aq. methanol, gave sandaracopimaric acid (0·9 g), m.p. and mixed m.p. 167–169°. Further elution with CH_2Cl_2 gave oils while 2·5% and 5% acetone in CH_2Cl_2 gave imbricatolic acid as an oil (3·5 g), essentially homogeneous by TLC and GLC. Its i.r. spectrum showed ν_{max} (film) at 3080, 1640, and 888 cm^{-1} ($>C{=}CH_2$), and 1690 cm^{-1} (—COOH). It was converted to the methyl ester (CH_2N_2) and rechromatographed on silica gel (120 g) containing 5% of water. Increasing concentrations of CH_2Cl_2 in benzene gave only traces of oils while CH_2Cl_2 and 1% acetone in CH_2Cl_2 gave methyl imbricatolate as a colourless oil (2·7 g). A portion was distilled, b.p. 155°/0·01 torr, $[\alpha]_D + 55°$ (*c*. 1·0 in $CHCl_3$), ν_{max} (film) 3080, 1640, and 890 cm^{-1} ($>C{=}CH_2$), 3350 cm^{-1} (—OH), and 1720 cm^{-1} (—COOMe), NMR δ 0·54 (3H, C-20), 1·22 (3H, C-18), 3·63 (3H, ester methyl), *ca*. 3·63 (m, 2H, C-15), 4·53 and 4·85 (2 x 1H, broad singlets, C-17), mol. wt. 336 (M.S.). (Found: C, 74·98; H, 10·65. Calc. for $C_{21}H_{36}O_3$: C, 75·01; H, 10·71%.)

Imbricatadiol

Methyl imbricatolate (200 mg) in dry ether (15 ml) was added to $LiAlH_4$ (100 mg) in ether (20 ml) and the mixture refluxed for 4 hr. Saturated aq. Na_2SO_4 was added followed by anhydrous Na_2SO_4. Filtration and evaporation gave a crystalline residue. Crystallized from acetonitrile this gave imbricatadiol (140 mg, 76%), m.p. 111–112° undepressed on admixture with an authentic sample (which had the same m.p. on our apparatus), $[\alpha]_D + 27°$ (*c*. 1·1 in $CHCl_3$). The i.r. spectra (in $CHCl_3$) were superimposable. NMR δ 0·63 (3H, C-20), 0·94 (3H, C-18), 3·22–3·82 (m, 4H, C-15 and C-19), 4·47 and 4·77 (2 × 1H, C-17). Mol. wt. 308 (M.S.). (Found: C, 77·61; H, 11·97. Calc. for $C_{20}H_{36}O_2$: C, 77·87; H, 11·76%.)

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