anabaseine in the entire animal, although the median proboscis contributes less than 1% of the body weight.

Our initial pharmacological assays with compounds I–III have provided several important results. First, 2,3'-bipyridyl rivals nicotine itself as a crustacean convulsant agent, although it is at least 20 times less lethal to mice than nicotine. Secondly, anabaseine is a potent paralyzing agent upon both crayfish and mice. The relative order of effectiveness in the mouse lethality bioassay, anabaseine > nicotine ≫ 2,3; -bipyridyl, has also been observed on the isolated frog rectus muscle preparation (KEM, unpublished results). According to current concepts about the molecular structure requirements for binding to arthropod and vertebrate nicotinic synaptic receptors, both 2,3'-bipyridyl and anabaseine would be predicted to have very little activity upon cholinergic synapses relative to nicotine, since both are weak bases (the pKa's of their most basic nitrogens are 4.4 and 6.7, respectively) and consequently would be largely unionized at physiological pH[13–15]. We suggest that the selective toxicity of 2,3'-bipyridyl may reflect substantial differences between arthropod and vertebrate receptor binding requirements, the planar, uncharged 2-pyridyl substituent binding more readily to crustacean than vertebrate receptors. If such differences do exist between the ligand-binding requirements of arthropod and vertebrate cholinergic receptors, they could provide a molecular basis for designing more selective insecticides[16].

Initial pharmacological experiments with pure nemertelline indicate that it has only a very small crustacean paralytic activity (Figure 1) and a very weak (less than 2,3'-bipyridyl) contractural action upon the frog rectus. Since it is the most abundant pyridine in *A. angulatus*, we suspect that nemertelline may act in some other manner, perhaps as a repellent to predators, which we cannot detect by our paralytic and lethality bioassays.

The *A. angulatus* pyridyls have tobacco alkaloid counterparts–anabaseine resembles myosmine, nemertelline resembles nicotelline, 2,3'-bipyridyl also occurs in tobacco, and a methylbipyridyl has recently been discovered in cured tobacco leaves[17]. This is a remarkable example of convergent biochemical evolution between an animal and a plant group. We have recently isolated the 3 most abundant pyridyls in a related hoplonemertine species (*Amphiporus ochraceus*) and found that they are all distinct from the compounds described here. Apparently the order Hoplonemertinea contains many pharmacologically active pyridyls. We expect that some of these will become useful chemical tools for investigating the mechanisms by which nicotinoids affect the nervous system.

[13] I. YAMOMOTO, in *Advances in Pest Control Research* (Ed. R. L. METCALFE; Interscience, New York 1965), vol. 6, p. 231.
[14] R. B. BARLOW and J. T. HAMILTON, Br. J. Pharmac. *18*, 543 (1962).
[15] K. SCHOFIELD, *Heteroaromatic Compounds: Pyrroles and Pyridines* (Plenum Press, New York 1967), p. 148.
[16] M. E. ELDEFRAWI, A. T. ELDEFRAWI and R. D. O'BRIEN, Agric. Food Chem. *18*, 1113 (1970).
[17] A. H. WARFIELD, W. D. GALLOWAY and A. G. KALLIANOS, Phytochemistry *11*, 3371 (1972).

The Structure of 4-Ketocedrol

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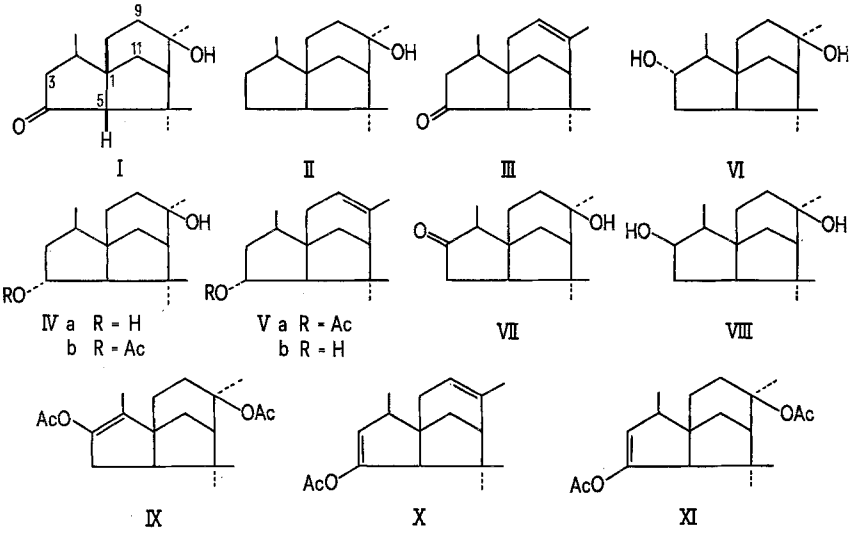
Summary. 4-Ketocedrol was isolated from *n*-hexane extraction of *Juniperus squamata* Linn. and its formula established by physical and chemical evidence.

Material and methods. The neutral fraction of a *n*-hexane extract of wood of *Juniperus squamata* Lamb. was investigated. Besides α-cedrol and its derivatives, 8S, 14-cedrandiol, a new keto-derivative I was isolated by chromatography on neutral alumina[2]. This new derivative of cedrol was found to be 4-ketocedrol.

Results and discussion. I. m.p. 129–130°, $C_{15}H_{24}O_2$, $[\alpha]_D$ −21.5° (C. 1.4 in CH_3OH), exhibits IR-absorption bands at 3460 (—OH) and 1730 cm^{-1} (C=O). It shows NMR-spectrum signals at τ 9.08 (3H, d, J = 6.6 Hz, $=CHCH_3$), 9.03 and 8.62 (each of 3H, s, $=C(CH_3)_2$), and 8.75 (3H, s, $=C(OH)CH_3$). The structure of compound I was suggested by the similarity of its NMR-spectrum with that of α-cedrol II. When I was subjected to Huang-Minlon modification reduction, α-cedrol was obtained. From this result I is a ketocedrol. I gave III by heating in 99% formic acid at 75–80°. The lqiuid III exhibited an isolated carbonyl group (1730 cm^{-1}) and a trisubstituted double bond (1645, 850 cm^{-1}) in IR-absorption bands, no maximum absorption above 210 nm in UV-spectrum, and NMR-spectrum signals at τ 8.27 (3H, br s, CH_3—C=C—H), and 4.62 (1H, m, CH_3—C=C—H). By these data, the location of the carbonyl at C_9 and C_{10} in I can be excluded. Sodium borohydride reduction of I in MeOH gave IVa as the sole product, m.p. 143–144°, ν_{max} 3200 cm^{-1} (—OH); its NMR-spectrum exhibits signal at τ 5.74 (1H, m, $W_{1/2}$ = 13 Hz, =C(OH)H). Treatment of diol IVa with acetic anhydride in pyridine yielded a monoacetate IVb, m.p. 68–70°, ν_{max} 1725, 1715 and 3270 cm^{-1}. The NMR spectrum of this acetate shows signals at τ 7.95 (3H, s, CH_3COO—) and 4.77 (1H, m, $W_{1/2}$ = 14 Hz, =C(OAc)H). When IVb was treated with formic acid at 75–80° it gave Va, ν_{max} 1730, 3040 and 805 cm^{-1}; with NMR-signals at τ 7.93 (3H, s, CH_3COO—) and 4.73 (1H, m, $W_{1/2}$ = 12 Hz, =C(OAc)H). Vb, m.p. 82–85°, saponification product from Va, shows a multiplet centered at τ 5.61 ($W_{1/2}$ = 13 Hz) in the NMR-spectrum. Examination of the coupling pattern of the proton attached to the carbon atom carrying the hydroxyl or acetyl group (with great $W_{1/2}$ value) in IVa, IVb, Va or Vb, excludes the carbonyl at C_{11} in I. Therefore the

[1] The authors are indebted to National Council on Science Development of China for financial support.
[2] The separation procedure will be published elsewhere.

carbonyl group must be located at C_3 or C_4. Recently, microbial hydroxylation of cedrol has been reported[3]. Three metabolites, 3α-hydroxycedrol VI, 3-ketocedrol VII and 3β-hydroxycedrol VIII, were isolated and identified. The proof of oxidation at C_3 was demonstrated by the preparation of IX from VII[4]. I and VII were obviously different compounds by comparison of their physical data. Therefore the carbonyl group of I must be located at C_4. This assignment was further supported when I gave X and XI by treatment with *p*-toluenesulphonic acid in isopropenyl acetate at 96°. The minor product X exhibits IR-absorption bands at 1755 and



1695 cm^{-1} (—C=C—OAc) and NMR-spectrum signals at τ 7.85 (3H, s, CH_3COO—) and 4.66 (2H, br s, CH_3—C=C—H, and AcO—C=C—H). The major product XI shows two acetate absorption bands in IR-spectrum at 1755 and 1725 cm^{-1} and NMR-spectrum signals at τ 8.03 and 7.85 (each of 3H, s, CH_3COO- and CH_3COO—C=C—) and 4.59 (1H, br s, CH_3COO—C=C—H). Finally, the assignment of the hydroxyl group at C_4 in IVa is in good agreement with the observation that on sodium borohydride reduction of I, the reducing agent approaches from the sterically less hindered β-side, thus giving exclusively IVa.

[3] K. C. WANG, L. Y. Ho and Y. S. CHENG, J. Chinese biochem. Soc. *1*, 53 (1972).
[4] Unpublished data (private communication from Dr. K. C. WANG).

Ascorbic Acid and Cholesterol: Effect of Graded Oral Intakes on Cholesterol Conversion to Bile Acids in Guinea-Pigs

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Summary. A significant correlation between liver ascorbic acid (AA) and total bile acids or liver bile acids has been established in guinea-pigs by direct determination of the bile acids, confirming an earlier hypothesis. The oxidation of cholesterol to bile acids is dependent on the AA status, but it cannot be further stimulated by AA when the animals are already on an adequate intake of the vitamin. This suggests that AA has a hypocholesterolaemic effect over a limited range of AA status.

In guinea-pigs with chronic ascorbic acid (AA)[1] hyposaturation, cholesterol accumulation in the liver was significantly enhanced and even more pronounced when feeding an atherogenic diet with added cholesterol resulting in significantly raised cholesterol levels in several tissues[2-4]. Catabolism of cholesterol was found to be decreased in scorbutic guinea-pigs, and addition of AA enhanced the conversion of (4-^{14}C) cholesterol to bile acids by liver mitochondrial preparations from AA-deficient guinea-pigs[5]. The rate of conversion of cholesterol to bile acids is significantly correlated with the hepatic AA concentration in guinea-pigs, as concluded from experiments on the catabolism of (26-^{14}C) cholesterol using $^{14}CO_2$ exhalation as an indirect measure of the formation of bile acids[6-9]. It was postulated that AA is essential in the hydroxylation of cholesterol and that the action of AA on its catabolism is mediated via cytochrome P-450[8]. The rate-limiting steps in the degradation of cholesterol are side-chain oxidation and 7α-hydroxyla-

[1] Abbreviations: AA, ascorbic acid; Na-AA, sodium ascorbate.
[2] E. GINTER, P. BOBEK, Z. KOPEC, M. OVECKA and L. CEREY, Z. Versuchstierk. *9*, 228 (1967).
[3] E. GINTER, P. BOBEK, J. BABALA and E. BARBIERIKOVA, Cor Vasa *11*, 65 (1969).
[4] E. GINTER, J. BABALA and J. CERVEN, J. atheroscler. Res. *10*, 341 (1969).
[5] R. GUCHHEIT, B. C. GULIA and N. C. GANGULI, Biochem. J. *86*, 193 (1963).
[6] E. GINTER, J. CERVEN, R. NEMEC and L. MIKUS, Am. J. clin. Nutr. *24*, 1238 (1971).
[7] E. GINTER, R. NEMEC and P. BOBEK, Br. J. Nutr. *28*, 205 (1972).
[8] E. GINTER, Science *179*, 702 (1973).
[9] E. GINTER, R. NEMEC, J. CERVEN and L. MIKUS, Lipids *8*, 135 (1973).