Recovery of 2,6-Dichloropurine.—The filter cake from a coupling run using 84 g of 2,6-dichloropurine was screened to remove molecular sieves and the remaining Celite mixture was suspended in water and brought to pH 11–12 with concentrated ammonium hydroxide. The basic solution was filtered (Celite) and the filtrate was brought to pH 5 by the addition of glacial acetic acid. Storage at ambient temperature for 16 hr caused the separation of 2,6-dichloropurine which after drying yielded a product (36.4 g) suitable for reuse in the nucleoside synthesis, mp 180–181° (lit.[8] 179–181°), $\lambda_{max}^{0.1\ N\ NaOH}$ 280 mμ (ε 8370), $\lambda_{max}^{0.1\ N\ HCl}$ 274 mμ (ε 8840).

Registry No.—1, 10147-12-3; 2, 10212-38-1.

(8) P. Bitterli and H. Erlenmeyer, *Helv. Chim. Acta*, **34**, 835 (1951).

The Structure of Echinacein, the Insecticidal Component of American Coneflower Roots

MARTIN JACOBSON

Entomology Research Division, U. S. Department of Agriculture, Beltsville, Maryland 20705

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The American coneflower, *Echinacea angustifolia* DC. and *E. pallida* (Nutt.) Britton (*Compositae*), is indigenous to Kansas, Nebraska, and Missouri. The roots are highly pungent when chewed[1] and are reported to contain a mosquito larvicide.[2]

In addition, echinacoside ($C_{35}H_{46}O_{20}$), which was isolated from the methanol extract of the roots by Stoll, *et al.*,[3] possesses antibacterial properties. The roots are available commercially and have been used medicinally in the healing of wounds and inflammations.

In 1954, we reported[4] the isolation and partial characterization of impure echinacein, an unstable sialogogue toxic to adult house flies, *Musca domestica* L., from the roots of *E. angustifolia*. Pure echinacein has now been isolated from these roots and from those of *E. pallida* by an improved procedure, and its complete structure has been determined.

Dried *E. angustifolia* roots[5] were extracted with pentane, and the active material was concentrated by partition with nitromethane. Distillation of the inactive pentane-soluble fraction gave a mixture of liquid hydrocarbons corresponding in physical properties to the substance $C_{15}H_{26}$ reported by Woods.[6] The nitromethane solution was purified to obtain the neutral fraction, which was chromatographed successively on neutral alumina and silicic acid to give impure echinacein, mp 63–64°. This material was purified with considerable difficulty by repeated crystallization from hexane, first at −78° and then at −10°, to obtain a 0.01% yield of pure echinacein (based on dry root) as a crystalline solid, mp 69–70°. When a trace of this material was placed on the tongue, it produced excessive salivation and an intense, burning, paralytic effect on the tongue and on the mucous membranes of the lips and mouth. Also it produced a high rate of knockdown and mortality in tests with adult house flies. Although pure echinacein was obtained in the same manner from dried roots of *E. pallida*, the yield was only 0.001%.

Attempts to identify echinacein were greatly hampered because the crystals are highly unstable and polymerize in air after 1 hr at room temperature and after 2 days in a nitrogen atmosphere at −10° (a natural antioxidant is apparently present in the crude extract of the roots). However, the active material is stable in a hydrocarbon solution at 5° for several months.

Analysis indicated formula $C_{16}H_{25}NO$ for echinacein, and experiments (hydrogenation, hydrolysis, oxidation, and iodine-catalyzed stereomutation) similar to those of Crombie[7] showed it to be N-isobutyl-*trans*-2,*cis*-6,-*trans*-8,*trans*-10-dodecatetraenamide and therefore identical with neoherculin and α-sanshool,[7] compounds obtained from plants of a completely unrelated family (*Rutaceae*).

Bohlmann and Grenz[8] have very recently reported the isolation from fresh roots of *E. angustifolia* and *E. purpurea* Moench. of the isobutylamides of *cis*-2,-*trans*-4-undecadien-8,10-diynoic and *cis*-2,*trans*-4-dodecadien-8,10-diynoic acids, as well as the presence of an inseparable mixture of the isobutylamides of 2,4,8,10-dodecatetraenoic acids. None of these compounds has been tested insecticidally.[9]

Experimental Section[10]

Isolation of Echinacein.—Ground root of *E. angustifolia* (8459 g) was extracted in a Soxhlet apparatus with pentane until no further color was removed (24 hr). The extract was concentrated to 1500 ml and extracted three times with 350-ml portions and twice with 200-ml portions of nitromethane. The combined nitromethane solution was freed of solvent under reduced pressure, the residue was taken up in 600 ml of ethyl ether, and the ether solution was washed thoroughly with water, 5% hydrochloric acid solution, 5% potassium hydroxide solution, and finally with water. After it was dried (Na_2SO_4), the ether solution of the neutral fraction was freed of solvent completely, leaving 35.6 g (0.42% of the root) of brown oil.

The nitromethane-extracted pentane solution was washed with water, dried (Na_2SO_4), and freed of solvent, and the residue was distilled to 100 g (1.2%) of a colorless, mobile liquid, bp 85° (0.5 mm), $n^{25}D$ 1.4488, that had no sialogogue or insecticidal effects.[6]

The brown oil was dissolved in a small amount of hexane and chromatographed on an alumina column (3.5 × 48 cm, Woelm neutral, activity grade I, purchased from Alupharm Chemicals, New Orleans, La.), by eluting it with 1:1 hexane–ether (1 l.). The eluate was freed of solvent, and the residue (17.4 g of viscous yellow liquid) was chromatographed on a silicic acid column (2.5 × 35 cm, Bio-Sil HA, minus 325 mesh, purchased from Bio-Rad Laboratories, Richmond, Calif.) by eluting first with 500 ml of benzene and then with 1 l. of benzene–ether (1:1). The benzene–ether eluate was freed of solvent under reduced pressure (20 mm) at 27°, and the semisolid residue (7.3 g) was triturated with seven 25-ml portions of hexane at room temperature. The combined pentane solution was concentrated to 20 ml under nitrogen and kept overnight at −10°; impure echinacein separated as a gel which was filtered off with difficulty

(1) H. Kraemer and M. Sollenberger, *Am. J. Pharm.*, **83**, 315 (1911).

(2) A. Hartzell and F. Wilcoxon, *Contrib. Boyce Thompson Inst.*, **12**, 127 (1941).

(3) A. Stoll, J. Renz, and A. Brack, *Helv. Chim. Acta*, **33**, 1877 (1950).

(4) M. Jacobson, *Science*, **120**, 1028 (1954).

(5) Obtained from S. B. Penick and Co., New York, N. Y. Reference to a company or product name does not imply approval or recommendation of the product by the U. S. Department of Agriculture to the exclusion of others that may be suitable.

(6) E. L. Woods, *Am. J. Pharm.*, **102**, 611 (1930). Our characterization of this material will be reported elsewhere.

(7) L. Crombie, *J. Chem. Soc.*, 995 (1955); L. Crombie and J. L. Tayler, *ibid.*, 2760 (1957).

(8) F. Bohlmann and M. Grenz, *Chem. Ber.*, **99**, 3197 (1966).

(9) F. Bohlmann, private communication.

(10) All melting points are corrected; boiling points are uncorrected. Analyses were carried out by Galbraith Laboratories, Inc., Knoxville, Tenn. Infrared spectra were determined with a Perkin-Elmer Model 521 spectrophotometer, and ultraviolet spectra were obtained with a Beckman Model DK-2 spectrophotometer.

and dried by pressing on a porous plate (1.2 g, mp 63–64°). Repeated crystallization of the crude product from hexane, first at −78° and then at −10°, gave 845 mg (0.01%) of pure echinacein as colorless needles: mp 69–70°; ultraviolet maxima (absolute ethanol) at 259, 270, and 279.5 mμ (ε 36,000, 47,000, 38,000); infrared absorption (KBr disk) at 3260 and 3070 (NH), 1668 (α-C=C), 1626 (C=O), 1559 (CONH), 944 (*cis*,-*trans*,*trans*-triene), and 972 cm^{-1} (*trans*-α-C=C).

Anal. Calcd for $C_{16}H_{25}NO$: C, 77.65; H, 10.20; N, 5.65. Found: C, 77.89; H, 10.29; N, 5.62.

The substance was a powerful sialogogue and caused rapid knockdown and high mortality when tested on adult house flies. It polymerized in the air after 1 hr at room temperature and after 2 days in a nitrogen atmosphere at −10° but could be kept unchanged for several months at 5° as a solution in hexane.

Echinacein was obtained in the same manner from the dried roots of *E. pallida*, but the total yield was only 0.001%.

Hydrogenation to N-isobutyllauramide[4] and permanganate oxidation[11] to give succinic, oxalic, and N-isobutyloxamic acids further characterized echinacein as the isobutylamide of 2,6,8,10-dodecatetraenoic acid.

Ultraviolet Stereomutation of Echinacein.—Echinacein (200 mg) was dissolved in 20 ml of hexane, a small crystal of iodine was added, and the solution was exposed to the direct light from an ultraviolet lamp for 2 hr while it was cooled with an electric fan to prevent evaporation. By the end of this period, the mixture had crystallized to a solid, waxy mass. It was melted by warming, 10 ml of hexane was added, and the mixture was heated to boiling on the steam bath and then allowed to cool. The mass of white crystals that separated was filtered off, washed with a little cold hexane, and recrystallized once from this solvent to give 180 mg of small, colorless needles, mp 112–116°.[12] The melting point remained unchanged after a second recrystallization.

Anal. Calcd for $C_{16}H_{25}NO$: C, 77.65; H, 10.20; N, 5.65. Found: C, 77.72; H, 10.18; N, 5.59.

The isomer showed ultraviolet maxima at 259, 268, and 278 mμ (ε 38,300, 48,500, and 38,900) and infrared bands at 3250, 3060, 1666, 1623, 1548, 996 (all-*trans*-triene), and 978 cm^{-1}.

Echinacein and its isomeride gave maleic anhydride adducts, mp 99–100° and 149–150°, respectively.

Registry No.—Echinacein, 504-97-2; all-*trans*-triene, 10076-00-3.

Acknowledgment.—The insecticidal tests were conducted by Mr. J. H. Fales, U. S. Department of Agriculture, Beltsville, Md.

(11) M. Jacobson, *J. Am. Chem. Soc.*, **73**, 100 (1951).

(12) Crombie and Tayler[7] reported mp 110–115° for the isomeride of α-sanshool.

Synthesis of Diterpenoid Acids. VI.[1] Conformations of Some Derivatives of Podocarpic Acid

A. E. LICKEI, A. C. RIEKE, AND D. M. S. WHEELER

Department of Chemistry, University of Nebraska, Lincoln, Nebraska 68508

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For other work,[1] we wanted to use the keto lactone (**1**) as an intermediate to podocarpic acid. We confirmed Bible's[2] result that **1** is transformed into **2a** in 80% yield by catalytic hydrogenation and found that the conversion can also be effected by lithium in liquid ammonia.[3] The thioketal (**4**) was prepared during unsuccessful attempts to remove the ketone at C_7.[6]

Compound **1** was synthesized[2,8] by heating the bromo ketone (**3b**)[9] with collidine. Earlier workers[8,10] suggested that the bromine is α (and thus **3c**). We now propose that the bromine is β and that ring B is distorted to a half-boat form. Our evidence is based mainly on nmr spectra taken in deuteriochloroform solutions and summarized in part A of Table I.

TABLE I[a]

Compd	C_4 methyl	C_{10} methyl	Ester methoxyl	Ring C methoxyl	C_5 proton	C_6 proton
			(A) $CDCl_3$ Solutions			
2b	76	62	218	224		
3a	75	66	220	229		
3b	92	51	222	230	148 (7)[b]	345 (7)[b]
1	80	67		232	138 (5.5)[b]	293 (5.5)[b]
4	80	77		227	127 (5.5)[b]	324 (5)[b]
			(B) Benzene Solutions			
2b	69	66	199	206		
3a	59	59	197	202		
3b	88	46	194	202	146 (7)[b]	366 (7)[b]
1	55	50		200	88 (6)[b]	266 (5.5)[b]
4	78	54[c]		202	86 (5)[b]	316 (5)[b]

[a] Chemical shifts in cycles per second (cps) from TMS. Spectra were measured on a Varian A-60 machine. [b] Figures in parenthesis are H_5–H_6 coupling constants. [c] This peak is a doublet with less than a 2-cps separation.

The small shift in comparison with steroid models[11] in the position of the C_{10} methyl group on going from **2b** to **3a** suggests that ring B is in a nonchair form. The fact that the 10 methyl in **3a** is not strongly shielded indicates that the methyl is not in the shielding cone of the ketone group; therefore, ring B has a flattened conformation (structure **6**, "sofa" in Chart I)[12] in which atoms 6–10 are approximately coplanar and atom 5 is out of the plane.[13]

Introduction of the bromine causes strong shielding of the C_{10} methyl and deshielding of the C_4 methyl. To account for the former, ring B in **3b** must be a half-boat. The deshielding of the C_4 methyl by the bromine (about that induced by a 1,3 diaxial interaction in a

(1) Part V: A. C. Ghosh, K. Mori, A. C. Rieke, S. K. Roy, and D. M. S. Wheeler, *J. Org. Chem.*, **32**, 722 (1967).

(2) R. H. Bible, personal communication.

(3) *Cf.* metal ammonia cleavage of hindered esters[4] and α-acetoxy ketones.[5]

(4) E. Wenkert and B. G. Jackson, *J. Am. Chem. Soc.*, **80**, 217 (1958).

(5) J. E. Starr, "Steroid Reactions," C. Djerassi, Ed., Holden-Day, Inc., San Francisco, Calif., 1963, p 299.

(6) Numbering in accord with the system suggested by McCrindle and Overton.[7]

(7) R. McCrindle and K. H. Overton, "Advances in Organic Chemistry, Methods and Results," Vol. 5, R. A. Raphael, E. C. Taylor, and H. Wynberg, Ed., Interscience Publishers, Inc., New York, N. Y., 1965, p 47.

(8) E. Wenkert, P. Beak, R. W. J. Carney, J. W. Chamberlin, D. B. R. Johnston, C. D. Roth, and A. Tahara, *Can. J. Chem.*, **41**, 1924 (1963).

(9) Melting points of 142–144.5° and 123–126° have been reported[2,8] for the bromo ketone. We find that the product of mp 128–130° is **3b** contaminated with a trace of **5**. The compounds are readily separated by preparative thin layer chromatography. Bromination of **3a** with pyridinium bromide perbromide is an effective method of obtaining **3b**.

(10) A. K. Bose, M. S. Manhas, and R. C. Cambie, *J. Org. Chem.*, **30**, 501 (1965).

(11) N. S. Bhacca and D. H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden Day, Inc., San Francisco, Calif., 1964, pp 19–21.

(12) E. M. Philbin and T. S. Wheeler, *Proc. Chem. Soc.*, 167 (1958).

(13) Wenkert and his co-workers[14] reached similar conclusions on the basis of a more complete study. They use the term "half-boat" to describe the normal conformation of ring B. However, it is clear from their discussion of totarol that they, like us, distinguish between nonchair conformations in which the 7-keto shields or does not shield the C_{10} methyl group.

(14) E. Wenkert, A. Afonso, P. Beak, R. W. J. Carney, P. W. Jeffs, and J. D. McChesney, *J. Org. Chem.*, **30**, 713 (1965).