Experimental Section[17]

Lithium Aluminum Hydride Reduction of 2 in Tetrahydrofuran.—A solution of **2** (0.54 g) in anhydrous tetrahydrofuran (30 ml) was heated at 50° with lithium aluminum hydride (0.30 g) for 24 hr. Excess hydride was destroyed with ethyl acetate and water. After filtration, the residue was washed with tetrahydrofuran, and the filtrate and washings were combined and concentrated to give a crystalline residue. Recrystallization from ethyl acetate gave methyl 2,4-di-*O*-methyl-β-D-galactopyranoside (**3**) (0.12 g), mp 166–169°, $[\alpha]^{25}D$ −4.8° (*c* 1.3, water). Smith[10] reported mp 165–166° and $[\alpha]D$ 0° (water) for methyl 2,4-di-*O*-methyl-β-D-galactopyranoside. The nuclear magnetic resonance (nmr) spectrum was consistent with that expected for **3**.

Anal. Calcd for $C_9H_{18}O_6$: C, 48.64; H, 8.16. Found: C, 48.87; H, 7.91.

Fractionation of the mother liquors on a silica gel column with ethyl acetate as eluent gave an additional 0.02 g of **3** and a faster moving component identified as methyl 3,6-anhydro-2,4-di-*O*-methyl-β-D-galactopyranoside (**4**) (0.06 g). This compound was purified by sublimation to give long white needles of mp 82°, $[\alpha]^{25}D$ −74° (*c* 0.7, water). Haworth, *et al.*,[18] reported mp 83° and $[\alpha]D$ −77° (water) for **4**.

2,4-Di-*O*-methyl-D-galactose.—A solution of **3** in 1.5 *N* sulfuric acid was heated at 95° for 6 hr, cooled, and passed through a column of Duolite A-4 (OH^-) ion-exchange resin. The resultant aqueous solution was concentrated to a syrup which on thin layer chromatography (tlc) (methyl acetate) showed some starting material as well as product. After chromatography on silica gel with methyl acetate as eluent, crystalline 2,4-di-*O*-methyl-D-galactose was obtained with mp 105–105.5°. An infrared spectrum of this compound was identical with that of authentic 2,4-di-*O*-methyl-D-galactose.[19]

Methyl 6-Deoxy-6-iodo-3-*O*-mesyl-2,4-di-*O*-methyl-β-D-galactopyranoside (**5**).—A solution of methyl 3,6-di-*O*-mesyl-2,4-di-*O*-methyl-β-D-galactopyranoside (**2**)[7] (1.6 g) and sodium iodide (1.3 g) in methyl ethyl ketone was boiled under reflux for 7 days. Sodium mesylate was removed by filtration and the filtrate concentrated to a syrup. The syrup was dissolved in chloroform and insoluble sodium iodide was removed by filtration. The filtrate was again concentrated to a yellow syrup (2.0 g) which was shown to be composed of one product plus starting material [tlc, chloroform–ethyl acetate (3:7)]. Fractionation of the mixture by silica gel column chromatography (same solvent system) gave **5** (1.1 g) and starting material (**2**) (0.6 g after recrystallization from ethanol). The product (**5**) was recrystallized from ethanol, mp 100–101.5°, $[\alpha]^{25}D$ +20° (*c* 1.5, chloroform). The infrared and nmr spectra were consistent with the proposed structure.

Anal. Calcd for $C_{10}H_{19}IO_7S$: C, 29.25; H, 4.65; I, 30.95; S, 7.80. Found: C, 29.55; H, 4.75; I, 30.58; S, 8.05.

Methyl 6-Deoxy-2,4-di-*O*-methyl-β-D-galactopyranoside (Methyl β-D-Labiloside) (**6**).—A solution of **5** (0.95 g) in anhydrous tetrahydrofuran (60 ml) was boiled under reflux with lithium aluminum hydride (0.70 g) for 7 hr. Excess lithium aluminum hydride was destroyed with ethyl acetate and water, and the residue was removed by filtration. The combined filtrate and washings were concentrated to yield a syrup which partially crystallized. Recrystallization from hexane gave **6** (0.12 g), mp 111°, $[\alpha]^{25}D$ −19° (*c* 1.3, chloroform).

Anal. Calcd for $C_9H_{18}O_5$: C, 52.41; H, 8.80. Found: C, 52.86; H, 8.97.[20]

6-Deoxy-2,4-di-*O*-methyl-D-galactose (Labilose) (**7**).—A solution of **6** (0.05 g) in 1 *N* sulfuric acid was heated at 95° for 3 hr, cooled, and neutralized with barium hydroxide. Barium sulfate was removed by filtration and the aqueous solution was concentrated to a syrup which was extracted with ether. Concentration of the ether extracts gave a syrup which crystallized on addition of hexane. Recrystallization from ether–hexane gave **7** (0.02 g), mp 131–134°, $[\alpha]^{25}D$ +94° (3 min) → +86° (final, water), $R_{glucose}$ 3.2 in 1-butanol–pyridine–water (10:3:3).

Anal. Calcd for $C_8H_{16}O_5$: C, 49.99; H, 8.39. Found: C, 50.25; H, 8.48.

Lithium Aluminum Hydride Reduction of 2 in Ether–Benzene.—A solution of **2** (0.54 g) and lithium aluminum hydride (0.30 g) in a mixture of ether (20 ml) and benzene (10 ml) was boiled under reflux for 24 hr. Tlc (ethyl acetate) indicated incomplete reaction. More lithium aluminum hydride (0.30 g) was added and the heating was continued for 48 hr. Tlc (ethyl acetate) now showed only methyl β-D-labiloside plus a trace of **4**. The product crystallized after the usual isolation procedure and was recrystallized from hexane to give **6** (0.18 g, 60%). This product was shown to be identical with **6** obtained by the previous procedure.

Registry No.—**7**, 10123-01-0; 2,4-di-*O*-methyl-D-galactose, 4301-53-1; **3**, 7801-09-4; **5**, 7801-10-7; **6**, 3006-40-4.

(17) All melting points are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Infracord spectrophotometer and nmr spectra were recorded on a Varian A-60 spectrometer. Silica gel column chromatography was performed on a silica gel, grade 950, 60–200 mesh from the Davison Co., Baltimore 3, Md. The microanalyses were done by Mr. C. DiPietro and the nmr spectra by Mr. F. H. Bissett, both of these laboratories.

(18) W. N. Haworth, J. Jackson, and F. Smith, *J. Chem. Soc.*, 620 (1940).

(19) The authors wish to thank Professor J. K. N. Jones for a sample of 2,4-di-*O*-methyl-D-galactose.

(20) Repeated vigorous drying of the sharp-melting, chromatographically pure material failed to remove all of the occluded hexane (recrystallization solvent) as demonstrated by the nmr analysis and the slightly high carbon analysis.

An Improved Synthesis of 9-β(-D-Arabinofuranosyl)-2-chloroadenine[1]

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The 9-β-D-arabinofuranosyl derivative of 2-chloroadenine (**1**) was previously prepared by Reist and Goodman[2] in an over-all yield of ~20% by the fusion of D-xylofuranose tetraacetate with 2,6-dichloropurine followed by preferential ammonolysis of the 6-chloro group and conversion to the 3′,5′-*O*-isopropylidene derivative of 2-chloro-9-β-D-xylofuranosyladenine which was subsequently methanesulfonated in the 2′ position and the isopropylidene blocking group removed by treatment with aqueous acetic acid. The resulting 2′-methanesulfonate on treatment with sodium methoxide yielded 2-chloro-9-(2,3-anhydro-β-D-lyxofuranosyl)adenine which was converted to **1** on warming with sodium acetate in dimethylformamide. It has been pointed out[2] that the above indirect method of synthesis with an acylated carbohydrate moiety was made necessary by the directing influences stated in the trans rule,[3] wherein use of an acylated arabinofuranose derivative would be expected to yield the undesired α anomer in this instance. Continued interest in the biological activity of **1** prompted the investigation of an adaptation of the Glaudemans and Fletcher[4] synthesis of 9-(β-D-arabinofuranosyl)adenine, wherein the directive influences of participating acyl groups on the sugar component in the nucleoside synthesis were obviated

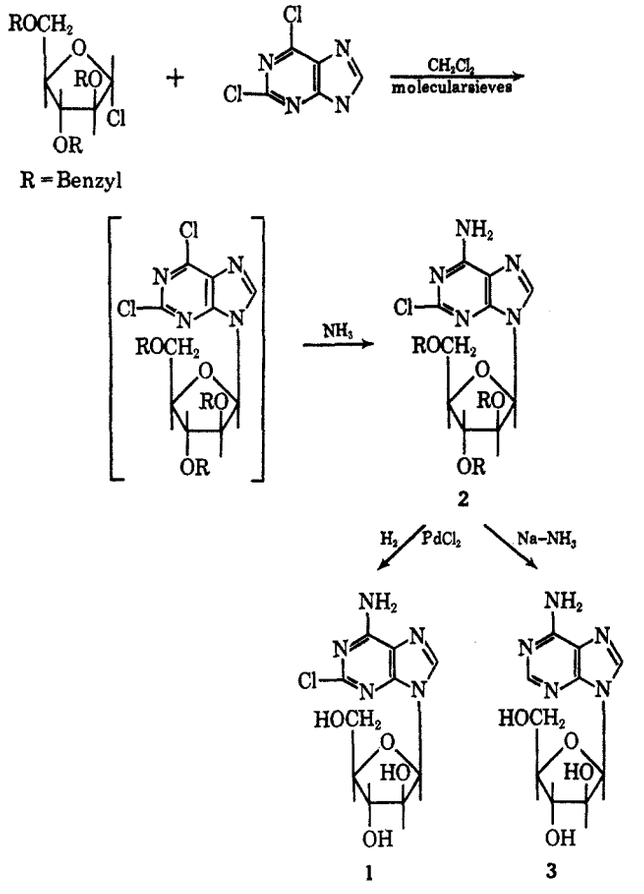
(1) This work was carried out under the auspicies of the Cancer Chemotherapy National Service Center, National Cancer Institutes, National Institutes of Health, U. S. Public Health Service, Contracts No. SA-43-ph-3764 and PH43-66-904. The opinions expressed in this paper are those of the authors and not necessarily those of the Cancer Chemotherapy National Service Center.

(2) E. J. Reist and L. Goodman, *Biochemistry*, **3**, 15 (1964).

(3) B. R. Baker, Ciba Foundation Symposium, Chemistry and Biology of Purines, Little, Brown and Co., Boston, Mass., 1957, pp 120–130.

(4) C. P. J. Glaudemans and H. G. Fletcher, Jr., *J. Org. Chem.*, **28**, 3004 (1963).

SCHEME I



by use of a partially benzylated arabinofuranose, these blocking groups being incapable of participation in the nucleoside formation. This convenient method of synthesis of a 1,2-*cis* purine nucleoside which was contemporary with the prior synthesis of **1** (Scheme I) has hitherto only been recorded as having been used in the original example.[4] By this procedure 2,3,5-tri-*O*-benzyl-α-D-arabinofuranosyl chloride and 2,6-dichloropurine were reacted in methylene chloride in the presence of molecular sieves for 1 week after the manner of Glaudemans and Fletcher.[4] The reaction mixture was filtered free of molecular sieves and unreacted 2,6-dichloropurine, and the crude residue remaining was aminated in the 6 position yielding 9-β-D-arabinofuranosyl-2-chloroadenine-2′,3′,5′-tribenzyl ether (**2**) as a well-defined crystalline solid in an over-all yield of 28%. Application of the sodium–liquid ammonia[5] debenzylation procedure resulted in concurrent reductive dehalogenation, and 9-(β-D-arabinofuranosyl)adenine (**3**) was the only compound isolated from the reaction mixture. Identity was confirmed by comparison with an authentic sample. Deblocking under acidic conditions using prereduced palladium chloride and hydrogen yielded **1** in 82% yield contaminated with a trace of **3** which was easily removed by recrystallization from water. The pure **1** obtained in an over-all yield of 19% proved identical with **1** prepared by the method of Reist and Goodman.[2] This alternate method of synthesis offers a relatively simple means of preparing **1** in laboratory quantities with over-all yields comparing favorably with those of the given method.[2]

(5) E. J. Reist, V. J. Bartuska, and L. Goodman, *J. Org. Chem.*, **29**, 3725 (1964).

Experimental Section

9-(β-D-Arabinofuranosyl)-2-chloroadenine-2′,3′,5′-tribenzyl Ether (**2**).—2,3,5-Tri-*O*-benzyl-1-*O*-*p*-nitrobenzoyl-D-arabinofuranose[6] (156 g, 0.274 mole) was dissolved in methylene chloride (1.8 l.) and the mixture was cooled to 0° while bubbling a slow stream of anhydrous hydrogen chloride through the reaction mixture (2.5 hr). The *p*-nitrobenzoic acid which had separated in quantitative yield was removed by filtration and washed with methylene chloride. The combined filtrate and wash was concentrated to dryness *in vacuo* (35° bath) and held at full pump vacuum (25°) for 16 hr. The residual oil was taken up in dry methylene chloride (1.5 l.) and 2,6-dichloropurine (110 g, 0.584 mole) and molecular sieves[7] (550 g) were added. The stoppered mixture was stirred for 1 week at ambient temperature. The coupling mixture was filtered through Celite and the filter cake was washed with a small amount of methylene chloride. The cake was reserved for recovery of unused 2,6-dichloropurine (see below) and the combined filtrate and wash was concentrated to dryness *in vacuo* (40° bath) yielding an oily semisolid (153 g). The residue was dissolved in methanol (600 ml), cooled to 0°, and saturated with anhydrous ammonia at 0°. The cold mixture was sealed in a bomb and allowed to stand at ambient temperature for 72 hr. At the conclusion of the amination the bomb was cooled in a Dry Ice mixture, opened, and allowed to equilibrate. The reaction residue was concentrated to dryness *in vacuo*, suspended in methylene chloride (1.2 l.), and washed successively with water containing a trace of acetic acid and water. The organic layer was dried over anhydrous magnesium sulfate and concentrated to dryness *in vacuo*. The residual oil was taken up in hot methanol (400 ml) and cooled slowly to give the crystalline product which was collected by filtration and washed with cold (−10°) methanol (43.7 g, 0.0763 mole, 28%), mp 133–135°, $[\alpha]^{24.6}D$ +44.0 (*c*, 0.5 $CHCl_3$) homogeneous on thin layer chromatography (tlc) (Sigel–MeOH–$CHCl_3$). An analytical sample was obtained by recrystallization from methanol containing a trace of acetone, mp 135–136°, $[\alpha]^{23.2}D$ +49.6° (*c*, 0.5 $CHCl_3$), $\lambda_{max}^{CHCl_3}$ 264 mμ (ε 14,300). The nuclear magnetic resonance (nmr) spectrum ($CDCl_3$–TMS internal standard) showed the same pattern as that of 9-(β-D-arabinofuranosyl)-adenine-2′,3′,5′-tribenzyl ether exclusive of the purine portion.

Anal. Calcd for $C_{31}H_{30}ClN_5O_4$ (572.1): C, 65.08; H, 5.29; Cl, 6.20; N, 12.24; O, 11.19. Found: C, 64.79; H, 5.14; Cl, 5.95; N, 12.30; O, 11.24.

9-(β-D-Arabinofuranosyl)-2-chloroadenine (**1**).—The above tri-*O*-benzyl nucleoside was deblocked under acidic conditions as follows. In a typical run palladium chloride (10 g) was prereduced in 2-methoxyethanol (100 ml) and the tribenzyl ether (11.7 g, 0.0205 mole) dissolved in 2-methoxyethanol (125 ml) was added. The mixture was hydrogenated at an initial pressure of 50 psig with the theoretical uptake being achieved in ~45 min. Excess hydrogen was bled from the system which was flushed with nitrogen. The catalyst was removed by filtration through a bed of Celite and the filter cake was washed with a small amount of 2-methoxyethanol. The combined filtrate and washings were neutralized by stirring with Dowex 2-X8 (HCO_3^-) ion-exchange resin. The neutralized solution, free of resin, was concentrated to dryness *in vacuo* and the residue was triturated with water and stored at 5° for 16 hr. The precipitated solid was collected by filtration, washed with cold water, and vacuum dried (80°). A further trituration with chloroform yielded nearly pure **1** (5.09 g, 0.0169 mole, 82%) contaminated with a trace of **3**. The crude **1** (28 g) was purified by two recrystallizations from hot water (4.5 l.); the hot solution being filtered through a thin bed of Celite Darco G-60. The purified product (23.1 g) was obtained as a shiny white crystalline solid, mp 242° dec (lit.[2] 239.0–240.5°), $[\alpha]^{25.0}D$ +6.9° (*c*, 0.29 pyridine). The material proved homogeneous in two paper chromatographic systems with R_{Ad} 1.1 (*n*-BuOH–H_2O) and R_{Ad} 1.4 (5% Na_2HPO_4) and was free of the trace of 9(β-D-arabinofuranosyl)adenine which was present prior to recrystallization. Infrared and ultraviolet spectra were identical with those of a sample prepared by the method of Reist and Goodman.[2] The nmr spectrum (dimethyl sulfoxide-d_6–tetramethylsilane internal standard) was identical with that of 9-β-D-arabinofuranosyladenine exclusive of the purine portion.

(6) R. Barker and H. G. Fletcher, *ibid.*, **26**, 4608 (1961).

(7) Linde, type 4A, 1/16-in. pellets.

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

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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.