pirated, and the residue crystallized from 30 ml. of 95% ethanol, yielding 0.37 g. (58%) of pale yellow needles, m.p. 172–174° dec. This material decolorized cold 2% permanganate solution. The analytical sample was prepared by recrystallization from absolute alcohol.

Anal. Calcd. for $C_{14}H_{15}ClN_2O_6S$: C, 44.86; H, 4.03; Cl, 9.46. Found: C, 44.80; H, 4.30; Cl, 9.29.

Attempted Preparation of 6-Chloro-3,4-dimethyl-Δ^3-cyclohexenyl 2′,4′-Dinitrophenyl Sulfide.—To a solution of 0.50 g. of II (R = H), in 25 ml. of benzene, was added 5 ml. 2,3-dimethyl-1,3-butadiene and the solution was refluxed 49 hours. Solvent and excess diene were aspirated, and the residue crystallized from carbon tetrachloride, giving 0.42 g. of orange needles, m.p. 129–131°. The mixed melting point with authentic starting material (m.p. 130–131°) was 129–131°. From the mother liquor, 0.05 g. more of material was recovered; total recovery 94%.

Treatment of II (R = C_6H_5) with Raney Nickel.—A solution of 1.50 g. (0.0036 mole) of 2-chloroethenyl 2′,4′-dinitrophenyl sulfide (II, R = phenyl),[2] in 75 ml. of absolute ethanol was refluxed with *ca.* 15 g. of Raney nickel for 1.5 hours. About 5 g. more of catalyst was added, and reflux continued for 1.5 hours. The mixture was filtered through diatomaceous earth, and the catalyst residue washed with two 25-ml. portions of boiling absolute ethanol. The washings were added to the filtrate, which was then concentrated in an air stream to about 100 ml. The solution became dark during this treatment, but was decolorized by adding 10 ml. of 6 *N* hydrochloric acid. Evaporation was continued almost to dryness, 25 ml. of water was added, and the precipitated solid removed by suction filtration. After washing with three 10-ml. portions of water, the solid was dried *in vacuo* and weighed 0.51 g. (100%). Decolorization with charcoal and crystallization from aqueous ethanol gave excellent, colorless plates melting at 48.5–49.5°. The Beilstein test for halogen was negative, and a mixed melting point with authentic 1,2-diphenylethane (m.p. 49–50°), prepared from stilbene, was 48.5–49.5°.

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Occurrence of Some Simple Sugars in Heartwood of Port Orford Cedar (*Chamaecyparis lawsoniana*)

BY GENE KRITCHEVSKY AND ARTHUR B. ANDERSON

RECEIVED FEBRUARY 14, 1955

Free arabinose and glucose appear to be common constituents in a great variety of both the heartwood and sapwood of the genus *Pinus*.[1] Aside from the isolation of L-arabinose from the heartwood of western red cedar (*Thuja plicata*),[2] very little appears to be known relative to the nature of some of the simple free carbohydrates present in the wood of various genera other than the *Pinus* species.

While investigating the water-soluble extract from Port Orford cedar heartwood (*Chamaecyparis lawsoniana*), a yield of 0.74 g. of a pentose sugar, identified as L(+)-arabinose was obtained from 500 g. of wood. This was the only sugar which was isolated in crystalline form. However, when the concentrated aqueous extract was submitted to paper partition chromatography, in addition to arabinose, the chromatograms showed R_f values which confirmed the presence of galactose, glucose and xylose. This appears to be the first report on the nature of some of the simple free sugars present in the genus *Chamaecyparis*.

Experimental

A composite sample of 500 g. of Port Orford cedar heartwood sawdust from five stumps was extracted in a glass percolator with five successive 1-liter portions of water at room temperature. The combined aqueous extract was concentrated on a water-bath at 40° and 18 mm. to 10 ml., then filtered to remove insoluble material. Approximately 1% of the filtrate was reserved for chromatographic analysis. The remainder of the filtrate was evaporated to dryness and the residue recrystallized from methanol, yielding 0.74 g. of a white crystalline product, m.p. 154–156° (0.15% yield based on the weight of wood used). Further recrystallization from hot methanol raised the m.p. to 159.3–160.2°, with an initial specific rotation of +136° (2% in water), and an equilibrium value of +104°. No change in melting point with an authentic sample of L(+)-arabinose with specific equilibrium rotation of +105.5°. A *p*-nitrophenyl hydrazone was prepared with m.p. 179.4–180°; mixed melting point with authentic phenylhydrazone derivative unchanged.

Paper chromatograms were run according to the method described by Partridge[3] on a portion of the above concentrated aqueous extract using four separate solvent mixtures. These solutions consisted of (1) ethyl acetate–pyridine–water (2–1–2),[4] (2) *sym*-collidine saturated with water,[5] (3) *n*-butyl alcohol–acetic acid–water (4–1–5)[5] and (4) isobutyric acid–water (4–1). Each of the chromatograms was run on a Whatman paper No. 1 (8 cm. × 57 cm.) using galactose, glucose, arabinose and xylose as the reference mixture. A chromogenic spraying agent of aniline oxalate[3] was used, which gives brown spots for the hexoses and pink spots for the pentoses.

R_f VALUES OBTAINED WITH EACH OF THE FOUR CHROMATOGRAMS

Solvent used	R_f values: Galactose	Glucose	Arabinose	Xylose	
Ethyl acetate–pyridine–water (2–1–2)	0.24	0.29	0.34	0.38	reference
	.24	.29	.34	.38	aq. extract
sym-Collidine satd. with H_2O	.35	.40	.44	.53	reference
	.35	.40	.44	.52	aq. extract
n-Butyl alcohol–acetic acid–water (4–1–5)	.16	.18	.22	.28	reference
	.16	.18	.22	.28	aq. extract
Isobutyric acid–water (4–1)	.14	.14	.19	.19	reference
	0.14		0.19		aq. extract

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The Site of Enzymatic Hydrogen Transfer in Diphosphopyridine Nucleotide[1]

BY FRANK A. LOEWUS, BIRGIT VENNESLAND AND DANIEL L. HARRIS

RECEIVED JANUARY 20, 1955

In a study of a model reaction for pyridine nucleotide dehydrogenases, Mauzerall and Westheimer[2] have shown that 1-benzyl-4-deuteriodihydronicotinamide transfers D to malachite green, whereas the 2-deuterio and 6-deuterio isomers do not. Their conclusion regarding the site of the reduction of the N-substituted nicotinamide was in agreement with the previous conclusions of Colowick and his collab-

(1) This investigation was supported in part from grants from the National Institutes of Health, United States Public Health Service, and by the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

(2) D. Mauzerall and F. H. Westheimer, THIS JOURNAL, **77**, 2261 (1955).

orators[3,4] who had shown that DPN (diphosphopyridine nucleotide) and its model compound, nicotinamide-1-methochloride are reduced at position 4 of the nicotinamide ring and not at position 2 or 6 as previously supposed.[5]

The present communication describes experiments in which the procedure of Mauzerall and Westheimer was extended to DPN. The results show that DPN containing deuterio-labeled nicotinamide behaves enzymatically like the labeled 1-benzylnicotinamide; *i.e.*, after reduction, D is transferred to an oxidant (pyruvate) from position 4 but not from position 2 or 6. The conclusion regarding the site of reduction of DPN is thus identical with that reached by Colowick and his collaborators, who used a quite different method.

Procedure and Results

The 2-, 4- and 6-deuterionicotinamides were provided by Dr. Mauzerall and Professor Westheimer, and their preparation has been described elsewhere.[2] These substances were incorporated into DPN by the enzymatic exchange reaction described by Zatman, *et al.*[6] The labeled DPN so prepared was reduced chemically with $Na_2S_2O_4$ in H_2O, and this reduced DPN was reoxidized enzymatically with pyruvate in the presence of lactic dehydrogenase. The lactate was diluted with carrier lactate, and converted to the phenacyl derivative which was analyzed for excess D.

Previous experiments have shown[7,8] that reduction of DPN with $Na_2S_2O_4$ in a medium of D_2O produces two diastereomers of monodeuterio reduced DPN, with a preponderance (*ca.* 65%) of that diastereomer which contains D on the side of the nicotinamide ring used specifically by lactic dehydrogenase. The reduced DPN used in the present experiments was prepared by reduction in H_2O, of a DPN already containing D in the nicotinamide moiety. In this case the DPN labeled with D in the reduced position should also be a mixture of two diastereomers, with a preponderance now of the diastereomer containing H on the side of the nicotinamide ring used by the enzyme. Only about 35% of the D in the reduced DPN should be transferred under these circumstances.

The results of the experiments are given in Table I. The second column gives the atoms of D per molecule of labeled nicotinamide. The third column gives the atoms of D found per molecule of DPN after the introduction of the labeled nicotinamide by exchange, and shows the extent of exchange achieved. Only partial exchange was obtained with 2-deuterionicotinamide, but with the 4- and 6-deuterionicotinamides, exchange was complete. In the latter two cases the observed D content of the DPN is actually somewhat higher than the observed theoretical maximum (*ca.* 85% of the D content of the nicotinamide). However, the discrepancies are not unreasonable in view of the relative inaccuracy of the determination of the D content of DPN.[7]

TABLE I

DEUTERIUM TRANSFER FROM LABELED DPN TO LACTATE

Location of D in nicotinamide	Atoms D per molecule of Nicotinamide	Atoms D per molecule of DPN[a]	Dilution factor of lactate	Atoms D per molecule of lactate Theory[b]	Atoms D per molecule of lactate Found
2-*d*	0.52	0.29	10	0.10[c]	0.00
4-*d*	.56	.55	27	.17[d]	.15
6-*d*	.80	.75	14	.26[d]	.00

[a] After exchange in the presence of DPNase. [b] Calculated on the assumption that 35% of the D in DPN should be transferred. [c] Calculated from the value in column 3. [d] Calculated on the assumption that the DPN contains 85% of the atoms of D per molecule of nicotinamide, as given in column 2.

The fifth column shows the theoretical atoms of D per molecule expected in the lactate, if 35% of the D in the DPN were transferred, and the last column shows the amount of D actually found in the lactate. It is clear that no detectable transfer of D occurred when the experiments were performed with DPN containing 2- or 6-deuterionicotinamide, whereas the DPN containing nicotinamide 4-*d* transferred the amount of D which would be expected if reduction occurred at position 4. As was to be expected, this finding also excludes any possible consideration of position 5 as the site of reduction.

Talalay, Loewus and Vennesland[9] have described elsewhere another type of experiment with DPN containing 4-deuterionicotinamide. In this case D was transferred from position 4 of the nicotinamide ring of DPN by a β-hydroxy steroid dehydrogenase which is stereospecific for the opposite side of the pyridine ring from that used by lactic dehydrogenase The procedure used for the preparation of the labeled DPN was the same as that described here. Talalay, *et al.*, also gave the result of an experiment with DPN labeled in position 6 (and possibly 5) as well as in position 4 of the nicotinamide ring. This DPN was prepared from a deuterionicotinamide furnished by Mauzerall and Westheimer and shown to contain 1.3 atoms of D per molecule.[10] In exchange and transfer experiments of the type described in the present paper the material was found to behave as though half of the D were located at position 4. These experiments gave results entirely compatible with those described here, but have not been given in detail, since they contribute no additional information.

Experimental

A typical experiment with 4-deuterionicotinamide is described below. The other experiments were conducted in similar fashion.

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(10) The multiply-labeled nicotinamide was prepared by first exchanging 4-carboxyquinoline in D_2O and then decarboxylating the 4-carboxyquinoline by boiling with benzoic acid prepared by hydrolysis of benzoic anhydride in D_2O. The deuterated quinoline thus obtained was oxidized to quinolinic acid with H_2O_2 in the presence of $CuSO_4$, and the quinolinic acid was decarboxylated to nicotinic acid which was converted to nicotinamide.

Incorporation of Nicotinamide-4-*d* into DPN.—One hundred and ten mg. of DPN (Pabst) was incubated at 37° for 3 hours in the presence of 100 mg. of nicotinamide-4-*d* with 5 ml. of beef spleen DPNase (*ca.* 750 units). After inactivation of the enzyme by heating the reaction mixture for 10 minutes in a water-bath at 70°, the mixture was cooled and centrifuged. The precipitate was washed with 5 ml. of H_2O and the combined supernatants were added to a Dowex-1 formate exchange column (20 × 1 cm.). The column was washed with 200 ml. of H_2O followed by 0.1 *M* formic acid which eluted the DPN. Seventy-seven mg. of material containing 68% DPN by enzymatic assay was recovered by lyophilization from the DPN-containing fractions. A portion of this DPN was analyzed for excess deuterium with glycine as a diluent.[7]

Reduction of DPN Containing Nicotinamide-4-*d*.—Forty mg. of the above material containing 27 mg. of pure DPN was reduced in 5 ml. of 1.3% $NaHCO_3$ solution with 30 mg. of $Na_2S_2O_4$.[11] After the reaction was complete, the mixture was pipetted into 15 ml. of absolute ethanol together with 0.5 ml. of wash water. After 20 minutes at −20°, the mixture was centrifuged free of precipitated salts. The latter were dissolved in 1 ml. of H_2O and the salts were reprecipitated with 3 ml. of absolute ethanol. The supernatants were combined, poured into 90 ml. of absolute ethanol and stored at −15° overnight. The resulting precipitate was centrifuged, washed with ether and dried *in vacuo*. The dried powder weighed 42 mg. and contained 18 mg. of reduced DPN by enzymatic assay.

Enzymatic Oxidation of Reduced DPN Containing Nicotinamide-4-*d*.—Thirty-one mg. of the reduced DPN (containing 13.2 mg. of reduced DPN by enzymatic assay) was oxidized with 0.18 ml. of 0.1 *M* sodium pyruvate in the presence of crystalline muscle lactic dehydrogenase in 5.0 ml. of 0.1 *M* phosphate buffer, *p*H 7.4. When the reaction was complete as indicated by the disappearance of the absorption band at 340 mμ, the enzyme was destroyed by heating for 1 minute in a boiling water-bath. The reaction mixture was cooled, adjusted to *p*H 1–2 with 6 *N* H_2SO_4 and 50.0 mg. of unlabeled lithium-L-lactate was added (26.9-fold dilution). The solution was centrifuged to remove denatured protein and the lactic acid was recovered by ether extraction and converted to its phenacyl ester which was analyzed for D, all as previously described.[8]

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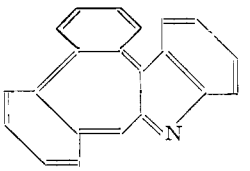
Azabenzazulenes. II. Attempted Preparation of Three Azatribenzazulenes and One Diazatribenzazulene

BY CHESTER W. MUTH, WEI-LIANG SUNG[1] AND ZINON B. PAPANASTASSIOU[1]

RECEIVED FEBRUARY 16, 1955

Studies on the preparation of 1-azatribenzazulenes are of interest because the additional benzene ring adds two double bonds for conjugation but is likely to make the tribenzo derivatives slightly more strained and much less nearly coplanar than the dibenzo derivatives.

The method used for the preparation of 1-azadibenzazulenes[2] was not successful for the preparation of either 1-azatribenz[b,e,g]azulene (I) or 1-azatribenz[b,f,h]azulene (II). The ketones used in the attempted preparations were, respectively, dibenzo[a,c][1,3]cycloheptadien-6-one (III) and dibenzo[a,c][1,3]cycloheptadien-5-one (IV). Ketone III was prepared with some improvements by a



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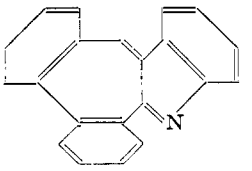


II

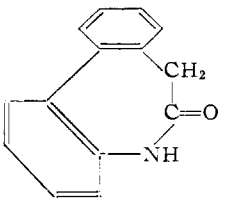
combination of the best reported methods[3a,b,c] in 5 steps in an over-all yield of 61%. Ketone IV was prepared in 14.5% over-all yield in ten steps by the method of Rapoport and Williams[4] except that 2′-cyanodiphenyl-2-carboxylyl chloride was obtained by the method of Bell.[5]

Ketone III was converted readily to 1,8-dihydro-1-azatribenz[b,e,g]azulene (V) by the method of Rogers and Corson.[6] However, ketone IV did not yield the expected indole, 1,8-dihydro-1-azatribenz[b,f,h]azulene (VI), when the foregoing method was tried. This failure was quite unexpected because 5H-6,7,8,9-tetrahydrocycloheptabenzen-5-one[2] did yield an indole with the foregoing method.

The dehydrogenation of V was unsuccessfully attempted by using (1) the chloranil[7] and (2) the palladium-on-charcoal[8] methods both of which had been used successfully for the preparation of 1-azadibenzazulenes.[2] Also unsuccessful for the dehydrogenation of V were (3) iodine and nitrobenzene,[9] (4) selenium in sealed tube[10] and (5) palladium-on-charcoal in sealed tube methods.

It should be noted that no benzazulene with a quinonoid structure has been reported and that 1-azatribenz[b,e,g]azaulene (I) would have an ortho-quinonoid structure in at least one benzene nucleus in each of the resonance forms.

Oxindole[11] and homophthalimide[12] both have a methylene group which will condense with benzaldehyde. However, it was found that the methylene group of the lactam of 2-amino-2′-biphenylacetic acid (VII) would not condense with benz-



VII

aldehyde, nitrosobenzene[13] or *p*-N,N-dimethylaminonitrosobenzene.[13] If the condensation reaction with VII had been successful, cyclization attempts to prepare azazulenes would have been made.

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