Anal. Calcd. for $C_{25}H_{34}O_6$: C, 69.74; H, 7.96. Found: C, 69.49; H, 7.92.

Δ^8-Allopregnen-3β-ol-7,11,20-trione Acetate (V).—A solution of 0.3 g. of the monoacetate IVa in 10 cc. of acetic acid was oxidized at room temperature for 2 hours with 0.15 g. of chromium trioxide in 5 cc. of 90% acetic acid. Dilution with water, filtration and purification of the precipitate by passage through a short column of alumina followed by recrystallization from hexane–benzene yielded 0.16 g. of the yellowish trione V with m.p. 171–173°, $[\alpha]^{20}D$ +50°, λ_{max}^{EtOH} 268 mμ, log ϵ 3.88.

Anal. Calcd. for $C_{23}H_{30}O_5$: C, 71.48; H, 7.82. Found: C, 71.63; H, 8.06.

The yield was raised to 70% when the oxidation was carried out with sodium dichromate[14] as described below for IX.

Allopregnane-3β,11α-diol-7,20-dione 3-Monoacetate (VIa).—A solution of 1.0 g. of the monoacetate IVa in 60 cc. of 95% ethanol was shaken for 2 hours with 100 mg. of pre-reduced 10% palladized charcoal catalyst (American Platinum Works, Newark, N. J.) in an atmosphere of hydrogen. Filtration of the catalyst, evaporation to dryness and recrystallization from hexane–acetone led to 0.88 g. of colorless plates with m.p. 184–186°, $[\alpha]^{20}D$ −10°, no selective absorption in the ultraviolet, λ_{max}^{nujol} 1736, 1718 and 1700 cm.$^{-1}$ and free hydroxyl band.

Anal. Calcd. for $C_{23}H_{34}O_5$: C, 70.74; H, 8.78. Found: C, 70.96; H, 8.85.

The diacetate VIb showed m.p. 155–157°, $[\alpha]^{20}D$ ±0°, $\lambda_{max}^{CS_2}$ 1736 cm.$^{-1}$ (acetate), 1718 cm.$^{-1}$ (7-ketone) and 1710 cm.$^{-1}$ (20-ketone)[13] but no free hydroxyl band. The infrared spectrum proved to be identical with that of an authentic specimen[6] prepared by an alternate procedure from 9α,11α-oxidoallopregnan-3β-ol-7,20-dione acetate.

Anal. Calcd. for $C_{25}H_{36}O_6$: C, 69.42; H, 8.39. Found: C, 69.51; H, 8.53.

Allopregnan-3β-ol-7,11,20-trione Acetate (VIc).—The oxidation of the monoacetate VIa was carried out exactly as described for the unsaturated analog IVa and proceeded in 80% yield to the desired trione VIc with m.p. 209–211°, $[\alpha]^{20}D$ +20°.

Anal. Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found: C, 71.25; H, 8.40.

Methyl Δ^8-3α-Acetoxy-7-keto-11α-hydroxycholenate (VIIIa).—Methyl Δ^8-3α-acetoxy-7-ketocholenate (VIIb)[3] (1.5 g.) was converted by the above described isopropenyl acetate procedure into its oily enol acetate (λ_{max}^{EtOH} 242 mμ, log ϵ 4.20, $\lambda_{max}^{CHCl_3}$ 1736 and 1728 cm.$^{-1}$ but no α,β-unsaturated carbonyl band), which was treated directly in chloroform solution at 5° with 1.2 moles of perbenzoic acid in the same solvent. The peracid consumption was practically complete after 72 hours at which time the solution was washed with sodium bicarbonate solution and water, dried and evaporated. Crystallization from methanol afforded 0.51 g. of colorless crystals with m.p. 170–172°, $[\alpha]^{20}D$ ± 0°, λ_{max}^{EtOH} 252 mμ, log ϵ 4.06, λ_{max}^{nujol} 1736, 1720 and 168 cm.$^{-1}$ and free hydroxyl band.

Anal. Calcd. for $C_{27}H_{40}O_6$: C, 70.40; H, 8.75. Found C, 70.15; H, 8.55.

The yield was not improved when the reaction was carried out with monoperphthalic acid at room temperature or in the ice-box.

Acetylation with acetic anhydride–pyridine on the steam-bath followed by recrystallization from acetone yielded the **diacetate VIIIb** with m.p. 158–160°, $[\alpha]^{20}D$ +48°, λ_{max}^{EtOH} 252 mμ, log ϵ 4.10, no free hydroxyl band in the infrared.

Anal. Calcd. for $C_{29}H_{42}O_7$: C, 69.29; H, 8.42. Found: C, 69.31; H, 8.55.

Methyl Δ^8-3α-Acetoxy-7,11-diketocholenate (IX).—A solution of 0.75 g. of the monoacetate VIIIa in 20 cc. of benzene was treated dropwise at 15° with 1.3 g. of sodium dichromate dihydrate in 20 cc. of glacial acetic acid and the mixture was let stand overnight. After dilution with water, extraction with ether, washing with sodium carbonate solution and water, the extract was dried, evaporated and crystallized from ether giving 0.53 g. of yellowish crystals of the unsaturated diketone IX with m.p. 119–120.5°, $[\alpha]^{20}D$ +36° (dioxane), λ_{max}^{EtOH} 270 mμ, log ϵ 3.94; reported,[3a] m.p. 115°, $[\alpha]^{20}D$ +36° (dioxane). A mixed melting point determination, kindly carried out by Prof. L. F. Fieser of Harvard University, confirmed the identity of the two specimens.

Anal. Calcd. for $C_{27}H_{38}O_6$: C, 70.71; H, 8.35. Found: C, 71.15; H, 8.41.

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[CONTRIBUTION NO. 246 FROM THE RESEARCH LABORATORIES OF HOFFMANN-LA ROCHE, INC.]

Esters of Basically Substituted 3-Pyridols with Physostigmine-like Activity[1]

BY ARTHUR STEMPEL AND JOHN A. AESCHLIMANN

RECEIVED DECEMBER 10, 1951

A series of twenty-four esters of 3-pyridol containing a basic substituent in the 2-position has been prepared in the form of the tertiary and quaternary salts. Several members of both series exhibit potent parasympathomimetic and anti-curare activity. The relationship of structure to activity is discussed.

Although physostigmine, the standard of natural parasympathomimetic drugs, is a monomethyl carbamyl ester of a phenolic tertiary amine, the first successful synthetic substitute, Prostigmin, owed its superiority to our finding that dimethylcarbamyl esters of phenolic *quaternary* ammonium compounds[2] combined pronounced parasympathomimetic action with stability. Salts of the corresponding weak tertiary bases exhibit comparatively low activity. Stedman[3] discovered the activity of monoalkylcarbamyl esters of quaternary phenols and synthesized Miotine which, like physostigmine, is a fairly strong tertiary base containing a monomethyl carbamyl group which is readily hydrolyzed in solution. This instability, along with its toxicity, prevents extensive use of physostigmine.

The preparation of quaternized carbamic esters of 3-pyridol exhibiting interesting anti-cholinesterase and parasympathomimetic activity has recently been reported from several laboratories.[4]

In a recent publication from this Laboratory,[5] the preparation of (3-hydroxy-2-pyridylmethyl)-

(1) Presented at XII International Congress of Pure and Applied Chemistry, New York, N. Y., Sept., 1951.

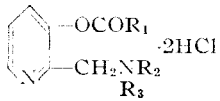
(2) (a) J. A. Aeschlimann and M. Reinert, *J. Pharm. Exp. Therap.*, **43**, 413 (1931); (b) J. A. Aeschlimann and A. Stempel, Barell Jubilee Vol., 306 (1946).

(3) (a) E. Stedman, *Biochem. J.*, **20**, 719 (1926); (b) A. C. White and E. Stedman, *J. Pharm. Exp. Therap.*, **41**, 259 (1931).

(4) (a) Hoffmann-La Roche A. G., Swiss Patents 246,834, 246,836, 252,486 (1947); (b) H. M. Wuest and E. H. Sakall, THIS JOURNAL, **73**, 1210 (1951); (c) R. D. Haworth, A. H. Lamberton and D. W. Woodcock, *J. Chem. Soc.*, 182 (1947).

(5) A. Stempel and E. C. Buzzi, THIS JOURNAL, **71**, 2969 (1949).

TABLE I

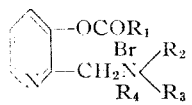


·2HCl

Ro 2-	R_1	R_2	R_3	M.p., °C.	Recrystallized from	Empirical formula	Analyses, % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N	Toxicity LD/50 mg./kg. (i.v.)	Anticholine esterase activity[a]	Anticurare activity[a]	Intestinal activity[a]
2126	$N(CH_3)_2$	CH_3	CH_3	164–167	EtOH–Et_2O	$C_{11}H_{19}O_2N_3Cl_2$	44.06	6.45	13.74	44.60	6.47	14.19	0.16	1	1/2	1/2
2126/1	$N(CH_3)_2$	CH_3	CH_3	128–130	*i*-PrOH–Et_2O	$C_{11}H_{18}O_2N_3Cl$[b]	[c]						0.16	1	1/2	1/2
2235	$N(CH_3)_2$	CH_3	H	140–141	MeOH–Et_2O	$C_{10}H_{17}O_2N_3Cl_2$	42.56	6.07		42.57	6.30		8.7		1/200	<1/100
2219	$N(CH_3)_2$	C_2H_5	C_2H_5	117–119	*i*-PrOH–Et_2O	$C_{13}H_{23}O_2N_3Cl_2$	48.15	7.15		48.11	7.18		0.17	1	1/4	1
2255	$N(CH_3)_2$	CH_3	$CH_2C_6H_5$	167–169	EtOH–Et_2O	$C_{17}H_{23}O_2N_3Cl_2$	54.84	6.23	11.29	54.89	6.02	11.25	3	<0.1	0	1.2
2256	$N(CH_3)_2$	NH		111–113	MeOH–Et_2O	$C_{14}H_{23}O_2N_3Cl_2$	50.00	6.89	12.50	49.94	6.76	12.51	0.075	0.1	0	1/7
2288	$N(CH_3)C_6H_5$	C_2H_5	C_2H_5	142–144	*i*-PrOH–Et_2O	$C_{18}H_{24}O_2N_3Cl$[d]	61.79	6.91	12.01	62.01	6.80	12.05	11.5	<0.1	0	1/15
2421	$N[CH(CH_3)_2]_2$	CH_3	CH_3	116–118	EtOAc	$C_{15}H_{27}O_2N_3Cl_2$	57.04	8.30	13.31	56.78	8.48	13.39	65	<<0.01	0	0

[a] Prostigmin = 1. [b] Monohydrochloride is hygroscopic. [c] Calcd.: N, 16.18; Cl, 13.65; Found: N, 15.83, 15.91; Cl, 13.38, 13.65. [d] Monohydrochloride.

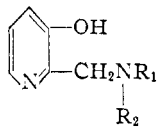
TABLE II



Ro 2-	R_1	R_2, R_3	R_4	M.p., °C.	Recrystallized from	Empirical formula	Analyses, % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N	Toxicity LD/50 mg./kg. (i.v.)	Anticholine esterase activity[a]	Anticurare activity[a]	Intestinal activity[a]
1658	$N(CH_3)_2$	CH_3	CH_3	175–177	EtOH–Et_2O	$C_{12}H_{20}O_2N_3Br$	45.29	6.34	13.21	45.55	6.09	12.97	0.075	1	1	1
1976	$N(CH_3)(C_6H_4Br\text{-}p)$	CH_3	CH_3	176–178	*i*-PrOH–Et_2O	$C_{17}H_{21}O_2N_3Br$	44.46	4.61	9.15	44.49	4.80	8.95	1.8	0.1	0.1	1/10
2007	$N(CH_3)(C_6H_4CH_3\text{-}p)$	CH_3	CH_3	153–155	MEK–Et_2O	$C_{18}H_{24}O_2N_3Br$	54.82	6.13	10.66	55.07	5.94	10.45	3.75	1/50	0	1/20
2043	$N[CH(CH_3)_2]_2$	CH_3	CH_3	173–175	EtOH–Et_2O	$C_{16}H_{28}O_2N_3Br$	51.34	7.54	11.23	51.40	6.96	11.48	22	0	0	0
2550	CH_3	CH_3	CH_3	166–167	MeOH–Et_2O	$C_{11}H_{17}O_2N_2Br$	45.68	5.93	9.69	46.10	6.01	9.13	113	<<0.01	0	1/500
2817	C_6H_5	CH_3	CH_3	191–193	MeOH–Et_2O	$C_{16}H_{19}O_2N_2Br$	54.71	5.45	7.98	54.55	5.25	7.90	31	>0.01	0	0
3128	$CH(C_6H_5)_2$	CH_3	CH_3	186–187	MeOH–Et_2O	$C_{23}H_{25}O_2N_2Br$	62.59	5.71	6.35	62.87	5.59	6.34	147 I.P.		0	
2016	$N(CH_3)_2$	C_2H_5	CH_3	141–143	EtOH–Et_2O	$C_{14}H_{24}O_2N_3Br$	48.56	6.99	12.14	48.24	6.97	12.18	0.07	>1	1	2
2400	$N(CH_3)_2$	C_2H_5	C_2H_5	166–169	MeOH–Et_2O	$C_{15}H_{26}O_2N_3Br$	50.00	7.27		50.18	7.29		0.2	<1	1/4	<1
2750	$N(CH_3)_2$	$C_3H_7\text{-}n$	CH_3	110–113	*i*-PrOH–Et_2O	$C_{16}H_{28}O_2N_3Br.H_2O$	48.98	7.54	10.71	49.34	7.38	10.68	.16	0.5	1/2–1	1/2
3124	$N(CH_3)_2$	$C_3H_7\text{-}i$	CH_3	159–161	Acetone–Et_2O	$C_{16}H_{28}O_2N_3Br$	51.34	7.54		51.38	7.48		.12	0.8	1/2	1/10
2279	$N(CH_3)_2$	$C_4H_9\text{-}n$	CH_3	154–155	*i*-PrOH–Et_2O	$C_{18}H_{32}O_2N_3Br$	53.73	8.02		53.73	8.08		.2	1	1/4	1.6
2227	$N(CH_3)_2$	$CH_2C_6H_5$	CH_3	177–179	EtOH–Et_2O	$C_{18}H_{24}O_2N_3Br$	54.82	6.13	10.66	54.53	6.38	10.75	1.5	<0.1	0	1/30
2253	$N(CH_3)_2$	NH[d]	CH_3	156–157	EtOH–Et_2O	$C_{15}H_{24}O_2N_3Br$	50.28	6.75		50.18	6.79		0.08	>0.1	1/4	1/2
2267	$N(CH_3)_2$			172–174	MeOH–Et_2O	$C_{22}H_{33}O_2N_4Br.CH_3OH$	55.52	7.50	11.26	55.24	7.65	11.16	1.9	>0.01	0	1/2
2112[b]	$N(CH_3)_2$	CH_3	CH_3	163–165	MeOH–Et_2O	$C_{13}H_{23}O_2N_3Br_2.H_2O$	36.21[c]	5.84	9.75	35.82	5.44	10.15	0.15	1	1/4	2

[a] Prostigmin = 1. [b] Bis-quaternary. [c] Calcd.: Br, 37.02. Found: Br, 37.29, 37.17. [d] R_2 = C_6H_5, R_3 = CH_2CH_2—$(CH_3)N(Br)$—$(C_2H_5)_2$.

TABLE III



R_1	R_2	B.p. °C.	Mm.	Yield, %	M.p. °C.	Analyses, % Calcd.[a] C	Calcd.[a] H	Calcd.[a] N	Found C	Found H	Found N
$C_3H_7(i)$	$C_3H_7(i)$	98–101	1.2	66	173–176[a]	51.24	7.89	9.96	51.24	7.16	10.05
									51.31	7.21	
C_6H_5	$CH_2CH_2N(CH_3)_2$				185–187[a]	55.81	6.73	12.21	55.88	6.82	12.23
		151–156	14	75	201–203[a]						
C_6H_5	CH_3			42	135–137[b]						
$C_6H_5CH_2$	H			71	236–241[a]	54.36	5.62	9.76	54.34	5.43	9.72

[a] Dihydrochloride.

amines by the Mannich reaction with 3-pyridol was described. These basically substituted 3-pyridols have been esterified with various acid chlorides and two series of compounds prepared with variations in the ester group and in the substituents on the side chain basic group. Esters of tertiary amines and their pharmacological properties have been listed in Table I and the quaternary salts in Table II.

In general, the (3-hydroxy-2-pyridylmethyl)-amines were esterified by warming with the desired carbamic acid chloride in pyridine solution and the esters then isolated as dihydrochlorides. The free esters usually reacted rapidly with alkyl halides such as methyl bromide to produce nicely crystalline monoquaternary ammonium salts.

Relationship between Structure and Activity

An examination of Table I indicates that the most potent basic esters are those in which the substituted pyridol is esterified with dimethylcarbamyl chloride and R_2 and R_3 are methyl or ethyl. The most potent members prepared are Ro 2-2126 and Ro 2-2219. These compounds, although tertiary bases, compare favorably with Prostigmin in anti-cholinesterase, anti-curare and intestinal stimulation action. They also have an oral toxicity closely approximating that found on intravenous administration indicating rapid absorption orally and are the first stable physostigmine analogs effective in as low dosage orally as the natural alkaloid. The duration of action, of Ro 2-2126 particularly as an anti-curare agent, proved to be greater in animal experiments than that of Prostigmin regardless of the route of administration. Replacing a methyl group with a benzyl group in the basic side chain gave a potent intestinal stimulant, Ro 2-2255, which was a weak choline esterase inhibitor and had no anti-curare action. Catalytic debenzylation of Ro 2-2255 produced the secondary amine, Ro 2-2235, which was devoid of these desirable properties.

The quaternary compounds, Ro 2-1658 and Ro 2-2016, corresponding to Ro 2-2126 and Ro 2-2219 are about as active by the parenteral route but much less potent when given orally. Among the quaternary salts listed in Table II, the dimethyl carbamyl esters were again the most active. When R_2, R_3 and R_4 were chosen from $CH_3C_2H_5$,C_3H_7-*n* and -*i*) and C_4H_9-*n*, the most potent anti-curare agents were formed. Combining R_3 and R_4 in a piperidine ring did not greatly lower the anti-curare and intestinal activity. These compounds, however, possess no particular advantage over Prostigmin. Replacement of a methyl group with a benzyl group greatly reduced all activity.

The substitution of the diisopropyl carbamyl group for the dimethyl carbamyl in active compounds such as Ro 2-1658 and Ro 2-2126 destroyed almost all of the desired activity.

All the salts described in Table II, with the exception of Ro 2-2112, were quaternized on only one of the two tertiary nitrogens available. In Ro 2-2112, quaternization under more drastic conditions produced a bis-quaternary ammonium compound. This did not greatly enhance the activity over that of the parent compound Ro 2-1658.

Position of the Quaternary Group

The structural formulas of the compounds in Table II indicate that there are two positions in which quaternization can take place. Reaction with one mole of an alkyl halide, such as methyl bromide, took place rapidly in the cold to give a high yield of a nicely crystalline monoquaternary ammonium salt. To introduce a second quaternary group required the use of higher temperatures.

Taking advantage of the known amine replacement reaction that phenolic Mannich bases undergo,[6] it has been possible to show that quaternization takes place on the Mannich basic group before reaction on the pyridine nitrogen. It has been assumed that quaternization of the basic ester would occur in the same manner.

Although there was no replacement reaction when 2-dimethylaminomethyl-3-pyridol was heated with amines, the corresponding trimethylammonium salt reacted readily under the same conditions It was therefore possible to prepare 2-piperidinomethyl-3-pyridol[5] in high yield by heating (3-hydroxy - 2 - pyridylmethyl) - trimethylammonium bromide with piperidine. This replacement would indicate that the side chain nitrogen was quaternized rather than the pyridine nitrogen. This reaction has proved to be of great value and has been extended to prepare a series of (3-hydroxy-2-pyridylmethyl)-amines with amines that do not undergo the Mannich reaction or might react differently, as in the case of primary amines. The compounds listed in Table III were prepared in this way.

(6) H. R. Snyder and J. H. Brewster, THIS JOURNAL, **70**, 4230 (1948); **71**, 1058 (1949).

Experimental

The first three of the following syntheses are representative of the preparation of compounds listed in Tables I and II.

Dimethyl Carbamate of (3-Hydroxy-2-pyridylmethyl)-dimethylamine Dihydrochloride.—A solution containing 43 g. of (3-hydroxy-2-pyridylmethyl)-dimethylamine in 30 cc. of pyridine and 32 cc. of dimethylcarbamyl chloride was heated on a steam-bath for 2 hours. Most of the pyridine was then removed by distillation *in vacuo*. The residue was dissolved in cold water, made alkaline with sodium hydroxide, and extracted with ether. The ether layer was washed with water and dried over anhydrous sodium sulfate. After removal of the ether by distillation, the residue was heated on a steam-bath under a vacuum of 1 mm. to remove traces of pyridine. The residue was dissolved in ether and saturated with anhydrous hydrogen chloride. A light brown crystalline solid separated. This was dissolved in hot ethanol, decolorized with charcoal, and crystallized on addition of anhydrous ether. After recrystallization from a mixture of ethanol and ether or from acetonitrile, the dihydrochloride melted at 163–167°; yield 47%.

Dimethyl Carbamate of (3-Hydroxy-2-pyridylmethyl)-trimethylammonium Bromide.—The esterification product obtained in the above example, after removal of traces of pyridine *in vacuo*, was dissolved in a cold acetone solution of methyl bromide. Crystals of the quaternary ammonium salt began to separate in a short time. After recrystallization from a mixture of ethanol and ether, the product melted at 175–177° dec.; yield 60%.

Phenylmethyl Carbamate of (3-Hydroxy-2-pyridylmethyl)-diethylamine Monohydrochloride.—To a solution of 10 g. of (3-hydroxy-2-pyridylmethyl)-diethylamine in 10 cc. of pyridine, there was added 10 g. of phenylmethylcarbamyl chloride. In about two minutes, the acid chloride dissolved with considerable evolution of heat and bubbling. The solution was cooled in water and kept for about 16 hours at room temperature. The large crystals of the monohydrochloride which formed were filtered and washed with pyridine and anhydrous ether. After recrystallization from a mixture of isopropyl alcohol and ether, the product melted at 142–144°; yield 52%.

(3-Hydroxy-2-pyridylmethyl)-diisopropylamine.—A mixture of 24.7 g. of (3-hydroxy-2-pyridylmethyl)-trimethylammonium bromide and 60 g. of diisopropylamine was stirred and refluxed. There did not seem to be any reaction at the reflux temperature. On heating the above mixture in a rocking autoclave at 125° for 2 hours, reaction took place. After boiling off excess diisopropylamine, the residue was extracted with benzene and the benzene layer then washed with water. The residue after removal of benzene, was distilled. This gave 13.7 g. of (3-hydroxy-2-pyridylmethyl)-diisopropylamine, b.p. 98–101° (1.2 mm.); yield 66%.

(3-Hydroxy-2-pyridylmethyl)-diisopropylmethylammonium Bromide.—A cold solution of (3-hydroxy-2-pyridylmethyl)-diisopropylamine in acetone containing methyl bromide gave crystals of the quaternary salt. After two crystallizations from a mixture of methanol and ether, it melted at 171–172° dec.; yield 82%.

Anal. Calcd. for $C_{13}H_{23}ON_2Br$: C, 51.49; H, 7.64; N, 9.24. Found: C, 51.19; H, 7.49; N, 8.96.

2-Benzylaminomethyl-3-pyridol Dihydrochloride.—On addition of 60 g. of benzylamine to 24.7 g. of (3-hydroxy-2-pyridylmethyl)-trimethylammonium bromide, the temperature rose to 33°. The reaction mixture was then stirred and heated at 50° for 1.5 hours, during which time the originally milky mixture became clear. After removal of excess benzylamine by distillation *in vacuo*, the residue was dissolved in 10% sodium hydroxide. The insoluble oil that separated was extracted with ether. On neutralization of the alkaline layer with hydrochloric acid to about *p*H 8, an oil separated that was extracted with benzene. The residue after removal of benzene was dissolved in ethanol and alcoholic hydrogen chloride added. The crystalline dihydrochloride separated in 71% yield. After two crystallizations from methanol, it melted at 236–241°.

Acknowledgments.—The authors are indebted to the late Dr. G. Lehmann and Dr. L. Randall of the Pharmacology Dept., Hoffmann-La Roche, Inc., for the pharmacological data presented. We wish to thank Dr. A. Steyermark and his staff for the microanalyses reported.

NUTLEY 10, N. J.

[CONTRIBUTION NO. 255 FROM THE DEPARTMENT OF ORGANIC CHEMISTRY AND ENZYMOLOGY, FORDHAM UNIVERSITY]

Investigations on Lignin and Lignification. IX. The Relationship Between the Action of Brown Rot Fungi, Cellulose Degradation and Lignin Composition in Bagasse

BY GEORGE DE STEVENS AND F. F. NORD

RECEIVED FEBRUARY 28, 1952

A comparative study of the effect of four wood-destroying fungi of the "brown rot" type on the dissimilation of cellulose in bagasse has been carried out. The lignins liberated by the cellulolytic action of each of these molds have been characterized. Their identity with bagasse native lignin is discussed.

The importance of wood-destroying fungi of the "brown rot" type as agents of cellulose degradation for the isolation of additional amounts of unaltered lignin from woody tissues has been well established.[1,2] However, these molds reveal a certain amount of specificity with respect to the type of wood attacked and their rate of growth when cultivated on a wood species. That is, certain "brown rots" thrive better when softwoods are used as substrates, whereas others show optimum activity with hardwoods.[3] In a recent report from this Laboratory,[4] the isolation and characterization of the native lignin and enzymatically liberated lignin from bagasse, the supporting fiber from the annual plant, sugar cane, was described. Since bagasse cannot be assigned to either the softwood or hardwood class, the problem of the choice of the "brown rot" fungus which would give rise to the highest rate of cellulose depletion necessarily presented itself. The resolution of this problem is, in part, reported in this paper.

(1) W. J. Schubert and F. F. Nord, THIS JOURNAL, **72**, 977, 3835 (1950).

(2) S. F. Kudzin and F. F. Nord, *ibid.*, **73**, 4619 (1951).

(3) M. K. Nobles, *Can. J. Research*, **26C**, 281 (1948).

(4) G. de Stevens and F. F. Nord, THIS JOURNAL, **73**, 4622 (1951).

Experimental

The wood species investigated in this study was virgin bagasse, and the "brown rot" organisms employed to effect the decay were the softwood molds, *Poria vaillantii* and *Lentinus lepideus*, and the hardwood fungi, *Daedalea quercinia* and *Polyporus sulphureus*.

Sterilization and Inoculation of Bagasse Samples.—Ten gram samples of the virgin bagasse were weighed into each