fraction was hydrolyzed with sulfuric acid, and copper purines precipitated.[12]

The TCA-extracted tissue was washed twice with cold ethanol, and lipides removed by refluxing three times with 150-ml. portions of 3:1 ethanol–ether, and once with ether; dry weight 385 mg. Combined nucleic acids were obtained by hot sodium chloride extractions, maintaining *p*H 7–8.[13] Nucleates were precipitated with 3 volumes of cold ethanol, and washed with ethanol and ether; weight 39.1 mg.

RNA and DNA were separated by hydrolysis in 2 ml. of *N* sodium hydroxide at room temperature, followed by precipitation of DNA.[14] The DNA precipitate was redissolved in 1 ml. of 0.1 *N* sodium hydroxide, immediately reprecipitated, and washed with TCA. DNA was extracted from the precipitate with hot TCA,[15] the solution made 1 *N* with hydrochloric acid, and hydrolyzed 1 hour at 100°. The solution was evaporated to dryness and a portion used for paper chromatography. The RNA contained in the DNA supernatants (0.1 ml. gave a negative cysteine–sulfuric acid test[16]) was hydrolyzed in *N* hydrochloric acid at 100° for 1 hour, and copper purines precipitated.

Copper purines of the ASN and RNA fractions were separately treated with hydrogen sulfide,[12] and the purine solutions evaporated *in vacuo* several times to remove excess acid. The residues were taken up in 0.5 *N* hydrochloric acid and separated on a column of Dowex-50.[10]

Radioactivity Assays.—All samples were counted with a thin mica window Geiger–Mueller tube. Aliquots of fractions obtained during the isolations were oxidized to carbon dioxide by wet combustion,[17] and barium carbonate plates prepared by filtration on paper. Purine fractions obtained in the ion-exchange separation were often not sufficiently pure for direct combustion and assay. The most satisfactory procedure was preliminary separation of purines on the ion-exchange column, followed by paper chromatography of portions of each purine on 3-cm. strips of Whatman No. 1 paper in Wyatt's[18] isopropyl alcohol–2 *N* hydrochloric acid solvent. Discs 14 mm. in diameter were cut from the purine spots with a sharp cork borer, counted, and eluted with 3 ml. of 0.1 *N* hydrochloric acid. Eluates were read in the Beckman DU spectrophotometer against eluates of discs cut from blank strips run at the same time. The quantity of purine present was calculated from the extinction coefficients of Wyatt,[18] correcting for any non-purine absorption by reading at 300 or 310 mμ.[19]

TABLE I

INCORPORATION OF GUANINE-8-C^{14} IN *T. geleii*

	Specific activity, c.p.m./mg. C[a]	c.p.m./μg. C[b]
Guanine administered	12,700	35.2
Respiratory CO_2, 4 days	0	
Whole cells	15	
Medium	1	
Acid-soluble compounds[c]	18	
Lipide-free tissue	186	
Combined nucleic acids	1000	
Nucleic acid-free tissue	2	
ASN guanine		33.8
ASN adenine		30.8
ASN hypoxanthine[d]		27.2
RNA guanine		31.2
RNA adenine		31.3
DNA guanine		31.2
DNA adenine		29.8

[a] Counts/min./mg. carbon, counted as barium carbonate, corrected to infinite thickness. [b] Counts/min./μg. carbon, counted on filter paper discs; this procedure results in higher specific activities than those obtained with barium carbonate plates of the same area. [c] Cold TCA extract after removal of TCA by ether extraction. [d] Arising principally by enzymatic deamination of adenine during isolation.

Results

The data of Table I show that guanine-8-C^{14} was utilized to the same extent for all the purines of the various fractions, within the accuracy of the method. It is evident that *T. geleii* W. utilizes exogenous purine exclusively, and does not synthesize purine from smaller precursors; such synthesis would dilute the activity of the guanine administered. As further evidence for lack of synthesis, no activity was found in ASN purines, total nucleic acids or on paper strips of hydrolyzed RNA and DNA after giving 30 mg. of sodium formate-C^{14} (31,000 counts/min./mg. carbon). Low activities were found in respiratory carbon dioxide during the five-day growth period.

The assistance of Miss Marjorie Anastasia is gratefully acknowledged.

(12) G. H. Hitchings, *J. Biol. Chem.*, **139**, 843 (1941).
(13) R. B. Hurlbert and V. R. Potter, *ibid.*, **195**, 257 (1952).
(14) G. Schmidt and S. J. Thannhauser, *ibid.*, **161**, 83 (1945).
(15) W. C. Schneider, *ibid.*, **161**, 293 (1945).
(16) P. K. Stumpf, *ibid.*, **169**, 367 (1947).
(17) D. D. Van Slyke, J. Plazin and J. R. Weisiger, *ibid.*, **191**, 299 (1951).
(18) G. R. Wyatt, *Biochem. J.*, **48**, 584 (1951).
(19) R. D. Hotchkiss, *J. Biol. Chem.*, **175**, 315 (1948).

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The Syntheses of 5- and 6-Chloroacetovanillone[1]

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During a study of the oxidation of chlorite lignin with nitrobenzene and alkali, 6-chlorovanillin and 6-chlorovanillic acid were isolated.[3] In anticipation of the isolation of chloro analogs of other products of the nitrobenzene oxidation of lignin, the synthesis of 5- and 6-chloroacetovanillone were undertaken.

Attempted condensation of a methyl Grignard reagent with 6-chlorovanillonitrile acetate was unsuccessful, as were the many attempted oxidations of 1-(6-chloro-4-hydroxy-3-methoxyphenyl)-1-ethanol, a compound prepared by the condensation of 6-chlorovanillin with methylmagnesium iodide. Recourse was then had to the diazomethane method for the synthesis of acetophenones from benzaldehydes.

Reaction of 6-chlorovanillin acetate with diazomethane yielded as the main product the β-hydroxyketone formed from two moles of the aldehyde with one mole of diazomethane, namely, 1,3-bis-(4-acetoxy-6-chloro-3-methoxyphenyl)-3-hydroxy-1-propanone (I). Deacetylation under mild alkaline conditions caused dehydration and yielded 6,6′-dichloro-4,4′-dihydroxy-3,3′-dimethoxychalcone (II). The β-hydroxyketone (I) was dehydrated to the diacetate of the chalcone (II) by means of acetic anhydride in pyridine. Complete hydrolysis of I yielded 6-chlorovanillin and the desired 6-chloroacetovanillone. 5-Chloroacetovanillone was prepared in similar fashion by hy-

(1) A portion of a thesis submitted to Lawrence College in partial fulfillment of the requirements of The Institute of Paper Chemistry for the degree of Doctor of Philosophy. This work was carried out under the direction of I. A. Pearl.
(2) Kimberly–Clark Corp., Neenah, Wisconsin.
(3) J. E. Jayne, Dissertation, The Institute of Paper Chemistry, 1953.

drolyzing directly the original reaction mixture obtained by treating 5-chlorovanillin acetate with diazomethane.

The mechanism of the formation of the chalcone as an intermediate in these reactions was proved by repeating the reactions with unchlorinated compounds to obtain previously known products.

Experimental

All melting points are uncorrected.

1-(6-Chloro-4-hydroxy-3-methoxyphenyl)-1-ethanol.—6-Chlorovanillin[4] was treated with methylmagnesium iodide essentially according to the procedure employed by Roberti, York and MacGregor for the preparation of analogous vanillin derivatives.[5] The carbinol, after crystallization from benzene–petroleum ether (b.p. 65–110°), melted at 128–128.5°.

Anal. Calcd. for $C_9H_{11}O_3Cl$: CH_3O, 15.3. Found: CH_3O, 15.4.

6-Chlorovanillin Acetate.—6-Chlorovanillin was treated with acetic anhydride essentially according to the preparation of vanillin acetate by Pisovschi.[6] The product was crystallized from absolute ethanol to give white needles melting at 97–97.5°.

Anal. Calcd. for $C_{10}H_9O_4Cl$: CH_3O, 13.6. Found: CH_3O, 13.7.

1,3-Bis-(4-acetoxy-6-chloro-3-methoxyphenyl)-3-hydroxy-1-propanone (I).—The diazomethane,[7] generated from 6.8 g. of nitrosomethylurea, was introduced beneath the surface of a solution of 8.6 g. of 6-chlorovanillin acetate in 250 ml. of ether kept at 0°. After 1.5 hours at 0° and 0.5 hour at 20°, the ether and excess diazomethane were removed by distillation leaving 9.0 g. of crude product melting at 115–145°. Repeated crystallization from ethanol raised the melting point to 157–158°.

Anal. Calcd. for $C_{21}H_{20}O_8Cl_2$: C, 53.52; H, 4.28; CH_3O, 13.2. Found: C, 53.72; H, 4.38; CH_3O, 13.2.

6,6'-Dichloro-4,4'-dihydroxy-3,3'-dimethoxychalcone (II).—I (4 g.) was warmed with 30 ml. of 5% sodium hydroxide solution. The deep orange solution was cooled, saturated with carbon dioxide and extracted with chloroform. Concentration of the chloroform extract yielded a crude product which, after several crystallizations from benzene–petroleum ether (b.p. 65–110°) and dilute ethanol, melted at 198.5–200° and weighed 150 mg.

Anal. Calcd. for $C_{17}H_{14}O_5Cl_2$: C, 55.30; H, 3.82; CH_3O, 16.8. Found: C, 55.38; H, 3.97; CH_3O, 16.5.

The residue from the chloroform-extracted solution was dissolved in 5% sodium hydroxide, acidified with sulfur dioxide and extracted with ether; the ether extract was concentrated to yield an additional 170 mg. of the 6-chlorochalcone melting at 194–195°.

The ether-extracted bisulfite solution was acidified and boiled; 0.4 g. of 6-chlorovanillin was obtained melting at 167–169°.

6-Chloroacetovanillone.—A solution of 2.1 g. of (I) in 80 ml. of 15% potassium hydroxide solution was refluxed for 20 hours. The solution was saturated with sulfur dioxide and the precipitate removed by filtration. The filtrate yielded a crop of white crystals during several hours under reduced pressure; the crystals weighed 150 mg. and melted from 100–105°. Crystallization from dilute ethanol raised the melting point to 109–110°.

Anal. Calcd. for $C_9H_9O_3Cl$: C, 53.88; H, 4.52; CH_3O, 15.5. Found: C, 53.70; H, 4.57; CH_3O, 15.8.

The 4,4'-Diacetoxy-6,6'-dichloro-3,3'-dimethoxychalcone (III).—Approximately 0.5 g. of I was dissolved in 2 ml. of hot pyridine, and 4 ml. of acetic anhydride was added. After standing for 20 hours, the mixture was poured onto ice. The oil which formed soon solidified; it melted at 129 to 133°. Several crystallizations from 95% ethanol raised the melting point to 137.5–138.5°.

Anal. Calcd. for $C_{21}H_{19}O_7Cl_2$: C, 55.64; H, 4.00; CH_3O, 13.7. Found: C, 55.61; H, 4.05; CH_3O, 13.7.

(4) L. C. Raiford and J. G. Lichty, THIS JOURNAL, **52**, 4576 (1930).

(5) P. C. Roberti, R. F. York and W. S. MacGregor, *ibid.*, **72**, 5760 (1950).

(6) I. J. Pisovschi, *Ber.*, **43**, 2139 (1910).

(7) F. Arndt, *Org. Syntheses*, **15**, 3 (1935).

III (180 mg.) was warmed on the steam-bath for 10 minutes with 5 ml. of ethanol and 3 ml. of 5% sodium hydroxide and then neutralized with dilute sulfuric acid. Upon cooling, a crop of yellow needles was formed which weighed 150 mg. and melted at 202–203.5°. No depression was observed in a mixed melting point with the previously described II.

5-Chlorovanillin Acetate.—5-Chlorovanillin acetate was prepared in a manner similar to that described for the 6-chloro compound. Crystallization from petroleum ether (b.p. 30–60°) gave a product melting at 63–64°.

Anal. Calcd. for $C_{10}H_9O_4Cl$: CH_3O, 13.6. Found: CH_3O, 13.8.

5-Chloroacetovanillone.—A large excess of diazomethane prepared from 45 g. of nitrosomethylurea was added to an ether solution of 7.15 g. of 5-chlorovanillin acetate at room temperature. The reaction mixture stood for several weeks. The residue was boiled briefly with 5% sodium hydroxide causing a portion of the oil to dissolve. Saturation of the alkaline solution with sulfur dioxide and extraction with ether gave an extract which was concentrated and applied to a column of acid-washed Magnesol.[8] The chromatogram was developed with 100:1 benzene–ethanol; elution of the major zone with acetone yielded a product melting at 122–125°. Its analysis and the analogy to the vanillin–diazomethane reaction showed it to be 5-chloroacetovanillone in 23% of the theoretical yield based on 5-chlorovanillin acetate. Treatment with charcoal and crystallization from dilute ethanol raised the melting point to 124–125°.

Anal. Calcd. for $C_9H_9O_3Cl$: C, 53.88; H, 4.52; CH_3O, 15.5. Found: C, 54.01; H, 4.53; CH_3O, 15.4.

6-Chlorovanillonitrile.—6-Chlorovanillonitrile acetate, (5.0 g.) was treated with methylmagnesium iodide; decomposition of the complex yielded 3.2 g. of crude 6-chlorovanillonitrile. Treatment with charcoal and crystallization from benzene gave a product melting at 151.5–152°.

Anal. Calcd. for $C_8H_6O_2ClN$: CH_3O, 16.9. Found: CH_3O, 16.7.

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(8) I. A. Pearl and E. E. Dickey, THIS JOURNAL, **73**, 863 (1951).

(9) L. C. Raiford and D. J. Potter, *ibid.*, **55**, 1682 (1933).

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Kinematic Viscosity of *n*-Heptane at Low Temperature

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The kinematic viscosity of *n*-heptane has been measured at several temperatures below 0°. The results at the lower temperatures are very appreciably different from the values tabulated by the American Petroleum Institute Project 44,[1] which we understand represent a long extrapolation of the previously available data. Because there is increasing interest in low temperature viscosities, it is hoped that this note will be of interest.

Experimental

All viscosities were measured in the Zeitfuchs cross arm-type capillary viscometer.[2] Temperatures were measured by a platinum resistance thermometer and were constant to ±0.01°. Four samples of *n*-heptane were used. The purity was established as 99.8 mole % or better by cooling curves. Viscometers were calibrated using water at 20° with an assumed value for the viscosity of water at this tempera-

(1) American Petroleum Institute Research Project 44, Selected Values of Properties of Hydrocarbons Table 20c-E (Part 2).

(2) J. F. Johnson, R. L. LeTourneau and Robert Matteson, *Anal. Chem.*, **24**, 1505 (1952).