Compound VI, $C_{22}H_{16}N_2$.—When 3 g. (0.02 mole) of phenylglyoxal hydrate and 3.8 g. (0.02 mole) of benzamidine hydrochloride in 300 ml. of water were boiled for three hours, a gummy substance formed which was filtered from the hot solution and recrystallized three times from ethyl alcohol. The colorless needles obtained melted at 170–172°. The yield was less than 1%.

Anal. Calcd. for $C_{22}H_{16}N_2$: C, 85.70; H, 5.19; N, 9.09. Found: C, 85.83; H, 5.38; N, 9.18.

No structure could be assigned to fit the formula and which would explain its formation. It would appear that the compound is the result of reaction between decomposition fragments.

TABLE I

ABSORPTION SPECTRA CURVES (1-CM. CELL)

Plate	Compound	Formula	Wt. used, g.	Solvent, ml.	Curve
I	IV	$(C_{15}H_{12}ON_2)_x$	0.00277	50 1% KOH	1
I	II	$2(C_{15}H_{12}ON_2)\cdot C_4H_8O_2$	.00250	50 1% KOH	2
I	III	$C_{15}H_{14}O_2N_2\cdot HCl$	.00266	50 1% KOH	3
I	VI	$C_{22}H_{16}N_2$	.00338	50 abs. EtOH	4
II	I	$2(C_{15}H_{14}O_2N_2)\cdot C_4H_8O_2$	.00250	50 abs. EtOH	1
II	III	$C_{15}H_{14}O_2N_2\cdot HCl$	.00112	50 EtOH contg. 5 ml. concd. HCl	2
II	II	$2(C_{15}H_{12}ON_2)\cdot C_4H_8O_2$	.00198	50 abs. EtOH	3

Absorption spectra data were obtained using a Hilger E3 spectrograph, Hilger Sector photometer and Eastman Wrattan and Wainwright Panchromatic plates. An under-water spark served as a light source.

It may be seen that Curves II and III, Plate I, and Curve I, Plate II are identical. This clearly demonstrates the formation of Compound II when base is added to a solution of either Compound I or Compound III.

Summary

1. The reaction between benzamidine and phenylglyoxal has been studied and found to yield a 2,4-diphenyl-4-hydroxyglyoxaline (or 2,4-diphenyl-5-keto-dihydroglyoxaline).

2. Intermediate, unstable compounds have been isolated and studied, and formulas proposed for them.

3. Absorption spectra curves in the ultraviolet and visible have been obtained for the compound studied.

BOULDER, COLORADO RECEIVED JUNE 16, 1942

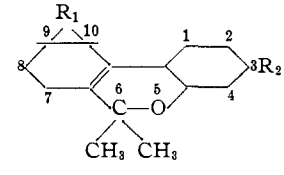
[CONTRIBUTION FROM THE WILLIAM G. KERCKHOFF LABORATORIES OF THE BIOLOGICAL SCIENCES, CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA, AND THE CHEMISTRY DEPARTMENT OF THE UNIVERSITY OF CALIFORNIA AT LOS ANGELES]

Some Analogs of Synthetic Tetrahydrocannabinol

BY GORDON A. ALLES, ROLAND N. ICKE AND GEORGE A. FEIGEN

The recent elucidation of the structure of cannabinol by Adams and co-workers,[1] and the discovery of marihuana activity in synthetic tetrahydrocannabinol[2a] and hexahydrocannabinol[2b] has opened the field for study of relationships between chemical constitution and this type of physiological action. The optically active tetrahydrocannabinols and hexahydrocannabinols derived by isomerization of cannabidiol[3] are of considerable interest in this connection, though their exact structure is in some doubt. Similarly, pulegone-5-*n*-alkylresorcinol products studied by Todd and co-workers,[4] and by Adams and co-workers[5] are of much interest, though the composition of such products is not yet certain.

Several series of compounds of known structure that are analogs or homologs of synthetic tetrahydrocannabinol and hexahydrocannabinol have been prepared by Adams and co-workers,[5,6] by Todd and co-workers[4,7] and by Bembry and Powell.[8] The object of the present work was to prepare and study the physiological activity of a series of analogs of synthetic tetrahydro*nor*-cannabinol[5,7,8] (Series I) and of tetrahydrocannabinol (Series II) that lack a hydroxyl group in the 1-position.



Series I, $R_1 = H$
Series II, $R_1 = CH_3$

The series of compounds were prepared in which the R_2 group in the 3-position was amyl, methyl, hydroxy, butyloxy, butyroxy, ethoxy and acetoxy. Of these, the hydroxy and acetoxy com-

(1) (a) Adams, Baker and Wearn, THIS JOURNAL, **62**, 2204 (1940); (b) Adams and Baker, *ibid.*, **62**, 2401 (1940).

(2) (a) Adams and Baker, *ibid.*, **62**, 2405 (1940); (b) Adams, Loewe, Pease, Cain, Wearn, Baker and Wolff, *ibid.*, **62**, 2566 (1940).

(3) (a) Adams, Pease, Cain and Clark, *ibid.*, **62**, 2402 (1940); (b) Adams, Cain, McPhee and Wearn, *ibid.*, **63**, 2209 (1941).

(4) Ghosh, Todd and Wright, *J. Chem. Soc.*, 137 (1941).

(5) (a) Adams, Smith and Loewe, THIS JOURNAL, **63**, 1973 (1941); (b) Adams, Loewe, Smith and McPhee, *ibid.*, **64**, 694 (1942).

(6) Adams, Loewe, Jelinek and Wolff, *ibid.*, **63**, 1971 (1941).

(7) (a) Ghosh, Todd and Wilkinson, *J. Chem. Soc.*, 1121 (1940); (b) Russell, Todd, Wilkinson, MacDonald and Woolfe, *ibid.*, 826 (1941).

(8) (a) Bembry and Powell, THIS JOURNAL, **63**, 2766 (1941); (b) Bembry, Columbia Univ. Dissertation (1941).

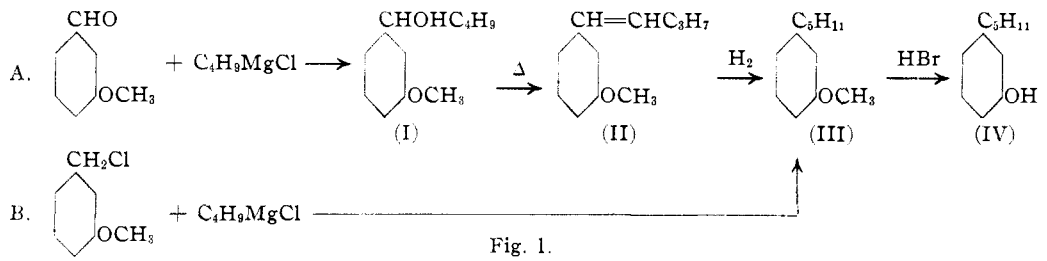


Fig. 1.

pounds had been previously prepared[7a] and reported to be inactive in the rabbit up to a dosage of 5 mg. per kg. intravenously, but were included in the present work for testing in dogs and extension of the rabbit testing. Synthetic tetrahydrocannabinol was prepared for use as a physiological test standard by making 1-hydroxy-3-*n*-amyl-9-methyl-7,8,9,10-tetrahydro-6-dibenzopyrone following the method of Adams and Baker,[1b] then converting this pyrone into the desired 6,6-dimethylpyran by the method of Ghosh, Todd and Wilkinson.[7a]

The synthesis of 3-*n*-amyl-6,6-dimethyl-7,8,9,10-tetrahydro-6-dibenzo-pyran (VI) and 3-*n*-amyl-6,6,9-trimethyl-7,8,9,10-tetrahydro-6-dibenzopyran (VIII) required the previously unknown 3-*n*-amylphenol (IV), which was prepared by two different routes, as shown in Fig. 1.

The amyl pyrones were prepared by the condensation of 3-*n*-amylphenol (IV) with the properly substituted cyclohexanone-2-carboxylate in accord with Sen and Basu.[9]

The butyl ethers of the hydroxy pyrones were prepared by two different methods as outlined in Fig. 2. The corresponding butyloxy pyrans were prepared both from the butyloxy pyrones and by direct butylation of the hydroxy pyrans.

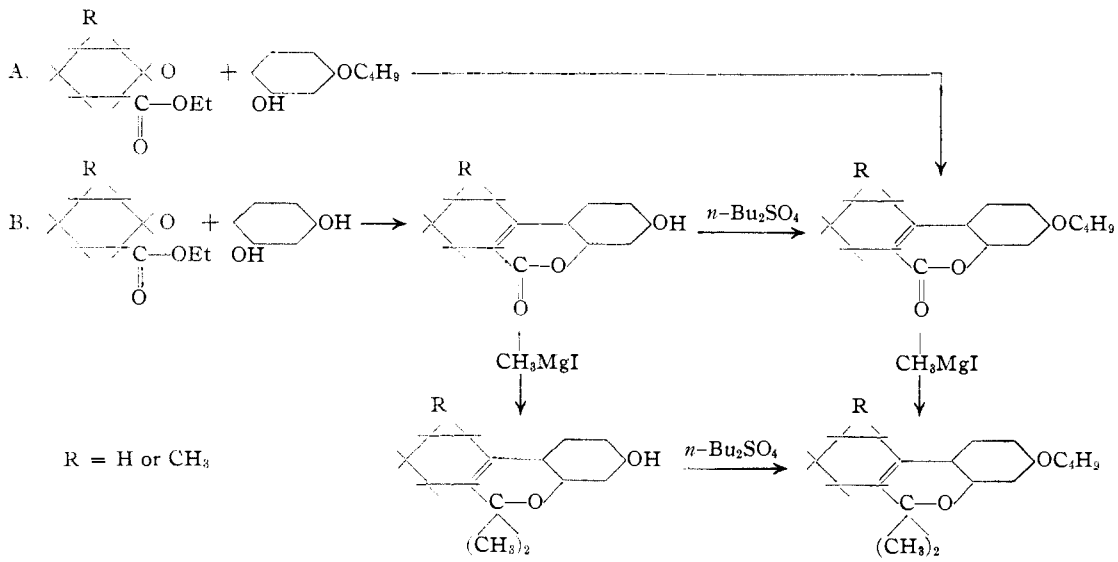


Fig. 2.

(9) Sen and Basu, *J. Indian Chem. Soc.*, **5**, 467 (1928).

Experimental

3-Methoxyphenyl-*n*-butylcarbinol (I).—A solution of 69.5 g. of 3-methoxybenzaldehyde in 150 ml. of ether was added slowly to a Grignard reagent prepared from 25 g. of magnesium and 101.5 g. of *n*-butyl chloride in 300 ml. of ether. After refluxing for one-half hour, the solution was poured into an excess of ice–sulfuric acid. On distillation, there was obtained 90.8 g. (92% yield) of a viscous, colorless oil, b. p. 115–125° (1 mm.). Redistillation gave, with very little loss, the product b. p. 128.5–129° (5 mm.), d^{25}_4 1.009.

Anal. Calcd. for $C_{12}H_{18}O_2$: C, 74.16; H, 9.34. Found: C, 74.2; H, 9.50.

1-(3-Methoxyphenyl)-amylene-1 (II).—A mixture of 54 g. of I and 10 g. of finely powdered potassium bisulfate was heated in an oil-bath at 135–160° for one hour. After all the water, which readily split out during the heating, had been removed, the product was distilled without further treatment, yielding 33.7 g. of a colorless liquid, b. p. 92–99° (1 mm.), d^{25}_4 0.985.

3-Methoxy-*n*-amylbenzene (III).—**A.**—A solution of 29.3 g. of II in 100 ml. of ethanol with 100 mg. of palladium oxide catalyst was reduced at room temperature under 3 atmospheres pressure of hydrogen, absorbing nearly the theoretical quantity in twenty minutes. After filtering off

the catalyst and distilling the ethanol, there was obtained 24.1 g. (81.5% yield) of a colorless liquid, b. p. 97–98° (3 mm.), d^{25}_4 0.947.

B.—3-Methoxybenzyl alcohol was prepared by Raney nickel and hydrogen reduction of the aldehyde at 90° under 90 atmospheres pressure. Treatment of 138 g. of the alcohol with 200 ml. of concentrated hydrochloric acid and 30 g. of anhydrous calcium chloride yielded 3-methoxybenzyl chloride, b. p. 75° (2 mm.), 78.3 g. of this in 100 ml. of dry benzene was added to a Grignard reagent prepared from 24.3 g. of magnesium, 92.5 g. of *n*-butyl chloride and 200 ml. of anhydrous ether. After distilling the ether and adding 200 ml. of dry benzene, the mixture was refluxed for thirty hours, then decomposed with ice–sulfuric acid. The benzene extract was separated, washed with dilute alkali, then water, and finally distilled to yield 15.2 g. of a colorless liquid, b. p. 96–99° (3 mm.), d^{25}_4 0.947.

Anal. Calcd. for $C_{12}H_{18}O$: C, 80.8; H, 10.17. Found: C, 79.6; H, 10.11.

Preparations A and B were demethylated separately and in each case the resultant phenol had the same properties.

3-*n*-Amylphenol (IV).—A mixture of 22 g. of III and 100 g. of 30% hydrobromic acid in glacial acetic acid was sealed in a bomb tube and heated for 3.5 hours in a boiling water-bath. The mixture became homogeneous during the heating period. After cooling, five volumes of water and about 5 g. of sodium bisulfite were added, then the solution was neutralized with sodium bicarbonate. Ether was added, the ether extract washed well with water, then the phenol extracted with excess 10% potassium hydroxide solution. Ether extraction of the alkali extract after acidification yielded 10 g. of a colorless viscous liquid, b. p. 103–108° (2 mm.). Upon redistillation this gave a main fraction, b. p. 99–100° (1 mm.), d^{25}_4 0.964. Yields by demethylating with constant boiling hydriodic acid were practically the same and the product identical.

Anal. Calcd. for $C_{11}H_{16}O$: C, 80.35; H, 9.81. Found: C, 79.94; H, 9.73.

The 3,5-dinitrobenzoate, recrystallized from isopropanol, melted at 70°.

Anal. Calcd. for $C_{18}H_{18}N_2O_6$: C, 60.5; H, 5.06. Found: C, 60.7; H, 5.12.

Excepting this dinitrobenzoate, all the preceding compounds proved to be difficult to analyze, since all had a tendency to explode during combustion.

3-*n*-Amyl-7,8,9,10-tetrahydro-6-dibenzopyrone (V).—To a mixture of 8.2 g. of IV and 8.7 g. of ethyl cyclohexanone-2-carboxylate cooled below 0°, was added slowly 40 ml. of concentrated sulfuric acid (also cooled to 0°), keeping the temperature below 25° by means of an ice-bath during the addition. The solution was allowed to stand for two hours in the cold-bath, poured onto crushed ice, and the salmon-colored viscous oil extracted with benzene. The benzene solution was washed with water, any unreacted phenol was extracted with 10% potassium hydroxide solution and washed again with water until the washings were neutral to litmus. After drying over magnesium sulfate, the benzene was removed, the unreacted ester (about 3 g.) was recovered under reduced pressure and, finally, the product was distilled under a mercury vapor pump vacuum (10 μ) with 180–185° oil-bath. A yield of 3.72 g. of pale yellow viscous liquid was obtained.

Anal. Calcd. for $C_{18}H_{22}O_2$: C, 79.96; H, 8.20. Found: C, 79.99; H, 8.20.

3-*n*-Amyl-6,6-dimethyl-7,8,9,10-tetrahydro-6-dibenzopyran (VI).—To a Grignard reagent prepared in the usual manner from 5.1 g. of magnesium and 30.2 g. of methyl iodide in anhydrous anisole was added a solution of 3.7 g. of V in 50 ml. of anisole. This solution was heated at 100° for eight hours with continuous mechanical stirring, cooled, poured onto ice containing 50 ml. of 12 *N* sulfuric acid and the anisole steam distilled. The residue was extracted with ether; the ether extract was washed with dilute sodium bicarbonate and then with water, dried over magnesium sulfate, and after removal of the solvent the product was distilled under 0.5 μ with a 140–145° bath, obtaining 3.5 g. (90% yield) of a pale yellow viscous liquid.

Anal. Calcd. for $C_{20}H_{28}O$: C, 84.45; H, 9.92. Found: C, 83.93; H, 9.86.

3-*n*-Amyl-9-methyl-7,8,9,10-tetrahydro-6-dibenzopyrone (VII).—This compound was prepared in the same manner as V from 10.8 g. of IV, 14.7 g. of ethyl 5-methyl-cyclohexanone-2-carboxylate, and 40 ml. of concentrated sulfuric acid. The yield was 5.9 g. of a viscous, pale yellow liquid, distilling at 3 μ with 200–205° bath.

Anal. Calcd. for $C_{19}H_{24}O_2$: C, 80.24; H, 8.51. Found: C, 80.28; H, 8.54.

3-*n*-Amyl-6,6,9-trimethyl-7,8,9,10-tetrahydro-6-dibenzopyran (VIII).—This compound was prepared by the same method as VI in 88% yield, distilling at 2 μ with a 155–160° bath.

Anal. Calcd. for $C_{21}H_{30}O$: C, 84.51; H, 10.13. Found: C, 84.50; H, 10.35.

3-Methyl-7,8,9,10-tetrahydro-6-dibenzopyrone (IX)[9] **and 3,9-dimethyl-7,8,9,10-tetrahydro-6-dibenzopyrone (X)** were prepared in the same manner as the amyl pyrones. Product X melted at 105–106°.

Anal. Calcd. for $C_{15}H_{16}O_2$: C, 78.93; H, 7.07. Found: C, 78.94; H, 7.60.

3,6,6-Trimethyl-7,8,9,10-tetrahydro-6-dibenzopyran (XI) and **3,6,6,9-tetramethyl-7,8,9,10-tetrahydro-6-dibenzopyran (XII)** were made by the same method as the amyl pyrans. XI distilled at 0.5 μ with a 100–105° bath.

Anal. Calcd. for $C_{16}H_{20}O$: C, 84.10; H, 8.83. Found: C, 83.80; H, 8.55.

XII distilled at 10 μ with 130–135° bath.

Anal. Calcd. for $C_{17}H_{22}O$: C, 84.24; H, 9.15. Found: C, 84.40; H, 9.11.

3-Hydroxy-7,8,9,10-tetrahydro-6-dibenzopyrone (XIII) and **3-hydroxy-9-methyl-7,8,9,10-tetrahydro-6-dibenzopyrone (XIV)** were made by Adams and Baker's[2a] modification of the method of Ahmad and Desai,[10] in yields of 86 and 80%, respectively.

3-Hydroxy-6,6-dimethyl-7,8,9,10-tetrahydro-6-dibenzopyran (XV) and **3-hydroxy-6,6,9-trimethyl-7,8,9,10-tetrahydro-6-dibenzopyran (XVI)** were made by the method of Todd and co-workers,[7a] except that the hydroxy pyrones, rather than the acetates, were converted into the pyrans.

(10) Ahmad and Desai, *J. Univ. Bombay*, **6**, Pt. II, 89 (1937).

3-*n*-Butyloxy-7,8,9,10-tetrahydro-6-dibenzopyrone (XVII)—A.—Ethyl cyclohexanone-2-carboxylate and resorcinol mono-*n*-butyl ether, obtained in 65% yield,[11] were condensed with phosphorus oxychloride in dry benzene.[2a] The product boiled at 240–243° under 3 mm. pressure and crystallized in the condenser. Crystallization from 95% ethanol gave fine white needles, m. p. 86–87°.

Anal. Calcd. for $C_{17}H_{20}O_3$: C, 75.02; H, 7.41. Found: C, 75.4; H, 7.31.

B.—Slightly more than 0.01 mole of di-*n*-butyl sulfate[12] was added to a mechanically stirred solution of 0.01 mole of XIII dissolved in 6 ml. of 2 *N* sodium hydroxide solution. After warming in an oil-bath at 90–110° for 1.5 hours, the mixture was allowed to cool slowly with continued rapid stirring. The excess sulfate was destroyed with concentrated ammonium hydroxide solution, and the precipitate was filtered off and crystallized from 95% ethanol in long, white, glistening needles. The yield was 2.2 g. of product, m. p. 87–88°. A mixture of this product with that from method **A**, m. p. 86–88°.

Anal. Calcd. (Same as **A**). Found: C, 74.4; H, 7.41.

3-*n*-Butyloxy-6,6-dimethyl-7,8,9,10-tetrahydro-6-dibenzopyran (XVIII).—To a Grignard reagent, prepared from 2.8 g. of magnesium and 16.5 g. of methyl iodide in 25 ml. of anisole, was added 4.5 g. of XVII. This solution was heated at 100° with continuous stirring for eight hours and then worked up in the same manner as VI. The yield was 3.4 g. of colorless viscous liquid distilling at 1 μ with a 133–134° bath.

Anal. Calcd. for $C_{19}H_{26}O_2$: C, 79.68; H, 9.15. Found: C, 79.52; H, 9.24.

3-*n*-Butyloxy-6,6,9-trimethyl-7,8,9,10-tetrahydro-6-dibenzopyran (XIX).—A solution of 2.44 g. of XVI in 10 ml. of 2 *N* sodium hydroxide solution was heated with 2.1 g. of di-*n*-butyl sulfate in an oil-bath at 90–110° for 1.5 hours with continuous mechanical stirring. The solution was cooled, extracted with ether, the extract washed well with water, and dried over magnesium sulfate. The yield was 2.9 g. of viscous yellow oil, distilling at 5 μ with 162–168° bath.

Anal. Calcd. for $C_{20}H_{28}O_2$: C, 79.96; H, 9.39. Found: C, 80.65; H, 9.15.

3-*n*-Butyroxy-6,6-dimethyl-7,8,9,10-tetrahydro-6-dibenzopyran (XX).—To 2.3 g. of XV was added 6.3 g. of *n*-butyric anhydride. Upon the addition of one drop of concentrated sulfuric acid, the hydroxypyran went into solution with a slight amount of spontaneous warming. After standing at room temperature for one hour the mixture was heated at 100° for 1.5 hours, cooled, considerable water added, and the organic layer separated. Any unreacted anhydride was hydrolyzed in accordance with Smith, Bryant and Mitchell's[13] pyridine–sodium iodide method. The resulting solution was cooled, diluted with 4 volumes of water and acidified with 4 *N* hydrochloric acid. The organic layer was separated with the aid of some ether, washed with water, and finally dried over magnesium sulfate. The yield was 2.1 g. of colorless, viscous liquid, distilling at 1 μ with 155–160° bath.

Anal. Calcd. for $C_{19}H_{24}O_3$: C, 75.97; H, 8.06. Found: C, 76.04; H, 8.49.

3-*n*-Butyroxy-6,6,9-trimethyl-7,8,9,10-tetrahydro-6-dibenzopyran (XXI).—This was prepared in the same way as XX from 2.44 g. of XVI and 6.33 g. of *n*-butyric anhydride. The yield was 2.6 g. of colorless, viscous liquid, distilling at 2 μ with 160–165° bath.

Anal. Calcd. for $C_{20}H_{26}O_3$: C, 76.40; H, 8.34. Found: C, 76.60; H, 8.56.

3-Ethoxy-6,6-dimethyl-7,8,9,10-tetrahydro-6-dibenzopyran (XXII).—This was prepared in the same manner as XIX, except that ethyl sulfate was used. The product, a pale yellow viscous liquid, was obtained in 91% yield, distilling at 5 μ with 120–125° bath.

Anal. Calcd. for $C_{17}H_{22}O_2$: C, 78.96; H, 8.56. Found: C, 78.32; H, 8.50.

3-Ethoxy-6,6,9-trimethyl-7,8,9,10-tetrahydro-7-dibenzopyran (XXIII).—This was also prepared by the same method as for XIX. The product, a viscous yellow liquid, was obtained in 78.6% yield, distilling at 10 μ with 145–150° bath.

Anal. Calcd. for $C_{18}H_{24}O_2$: C, 79.37; H, 8.88. Found: C, 78.70; H, 8.82.

3-Acetoxy-6,6-dimethyl-7,8,9,10-tetrahydro-6-dibenzopyran (XXIV) and **3-acetoxy-6,6,9-trimethyl-7,8,9,10-tetrahydro-6-dibenzopyran (XXV)** were prepared from XV and XVI by treatment with acetic anhydride and pyridine, yielding products corresponding to those of Todd and coworkers,[7n] which melted at 65–66° and 59–60°, respectively.

These pyrans that were synthesized in this work were tested for their marihuana activity in dogs by the method of Dixon.[14] Oral administration of each of the compounds of both Series I and II in doses of 50 and 100 mg. per kg. was tried, without the production of ataxia or the other symptoms of marihuana activity. In these same animals 8 mg. per kg. orally of synthetic tetrahydrocannabinol did produce notable ataxia.

The same pyrans were tested for their ability to produce corneal anesthesia in rabbits, following the method of Gayer[15] for testing of marihuana activity. Intravenous administration of each of the compounds of both Series I and II in 10% solution in acetone in doses of 10 and 20 mg. per kg. was tried, without the production of corneal anesthesia. These findings of inactivity in rabbits with regard to XV, XVI, XXIV and XXV extend the similar observations on these compounds made by Ghosh, Todd and Wilkinson.[7n] The testing of different preparations of synthetic tetrahydrocannabinol with doses of 1, 2, 4, 8, 16 and 32 mg. per kg. intravenously, each given to two different animals, did not cause any corneal anesthesia, though with the highest dosages there was some sluggishness of response. Ghosh, Todd and Wright[4] reported this compound to be active at 1 mg. per kg. intravenously in rabbits, and we are unable to explain the discrepancy between

(11) Klarmann, Gatyas and Shternov, THIS JOURNAL, **53**, 3404 (1931).

(12) "Organic Syntheses," **19**, 27 (1939).

(13) Smith, Bryant and Mitchell, THIS JOURNAL, **63**, 1700 (1941).

(14) Dixon, *Brit. Med. J.*, **2**, 1354 (1899); and *Pharm. J.*, 705 (1905).

(15) Gayer, *Arch. exp. Path. Pharmakol.*, **129**, 312 (1928).

these findings. Two different lots of synthetic tetrahydrocannabinol were prepared, and the rabbits used were shown to be responsive on subsequent days to fresh extracts of charas made with ethanol, evaporated, and taken up with acetone for testing.

We are indebted to Dr. C. E. Redemann for the analyses reported in this paper, and wish to thank him for many helpful suggestions made during the course of this work.

Summary

1. 3-*n*-Amylphenol has been prepared by two methods.

2. Derivatives of 6,6-dimethyl-7,8,9,10-tetrahydro-6-dibenzopyran substituted in the 3-position by *n*-amyl, methyl, hydroxy, butyloxy, butyroxy, ethoxy and acetoxy groups have been prepared.

3. Corresponding derivatives of 6,6,9-trimethyl-7,8,9,10-tetrahydro-6-dibenzopyran have also been prepared.

4. Neither of these two series of pyrans exhibits any significant degree of marihuana activity in dogs or rabbits.

PASADENA, CALIFORNIA RECEIVED MAY 12, 1942

[CONTRIBUTION FROM THE LABORATORY OF PHYSIOLOGICAL CHEMISTRY, UNIVERSITY OF MINNESOTA, MINNEAPOLIS]

The Optical Configuration of Glutamic Acid Isolated from Casein Hydrolyzates by Six Procedures[1]

BY JEANETTE C. OPSAHL AND L. EARLE ARNOW[2]

Kögl, *et al.*,[3,4,5] have claimed that the modification of the Foreman procedure employed by Chibnall, *et al.*,[6] isolates preferentially *l*(+)-glutamic acid, leaving much of the *d,l*-glutamic acid in the mother liquor. For example, they[5] isolated 2.472 g. of *l*(+)-glutamic acid from 23.4 g. of pig kidney protein by Chibnall's procedure. Two grams of *d,l*-glutamic acid then was added to the mother liquor, and the isolation was repeated. 1.290 g. of glutamic acid was isolated; the sample was found to contain 0.9335 g. of *l*(+)-glutamic acid and 0.3569 g. of *d*(−)-glutamic acid. The interpretation of this type of experiment is complicated by the possibility that the original isolation might not have been quantitative. In other words, the material recovered in the second isolation conceivably might have reflected more or less accurately the composition of the glutamic acid present in the mother liquor. Chibnall and his collaborators[6] isolated small amounts of *d,l*-glutamic acid from both normal and malignant tissue protein hydrolyzates by their procedure. However, they did not report experiments in which *d,l*-glutamic acid had been added to the hydrolyzate prior to isolation.

Graff, Rittenberg and Foster[7] added *d,l*-glutamic acid to protein hydrolyzates, and found that the material isolated by their modified Foreman procedure contained both optical forms of glutamic acid. However, they were investigating the optical composition of the glutamic acid in the hydrolyzates by means of an isotope (N^{15}) dilution method, and the percentages of *d*(−)-glutamic acid in the material actually isolated were not given in their paper.

It has been shown in several laboratories[8,9,10] that glutamic acid slowly racemizes in boiling hydrochloric acid solutions. Several reports describing the isolation of glutamic acid containing small percentages of *d*-isomer have been recorded.[6,11,12,13] This latter finding casts some doubt on the accuracy of the isotope dilution method as employed by Graff, *et al.*[7] If the figure reported by these workers for the *d*-isomer content of the glutamic acid of tissue protein hydrolyzates (not more than 0.5 ± 0.5%) is accepted, it then becomes necessary to assume that the methods

(1) The data presented in this paper were taken from a thesis submitted by Jeanette C. Opsahl to the Graduate Faculty of the University of Minnesota in partial fulfillment of the requirements for the M.S. degree.

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(3) F. Kögl and H. Erxleben, *Nature*, **144**, 111 (1939).

(4) F. Kögl, H. Erxleben and A. M. Akkerman, *Z. physiol. Chem.*, **261**, 141 (1939).

(5) F. Kögl and H. Erxleben, *ibid.*, **264**, 198 (1940).

(6) A. C. Chibnall, M. W. Rees, E. F. Williams and E. Boyland, *Biochem. J.*, **34**, 385 (1940).

(7) S. Graff, D. Rittenberg and G. L. Foster, *J. Biol. Chem.*, **133**, 745 (1940).

(8) L. E. Arnow and J. C. Opsahl, *ibid.*, **133**, 765 (1940).

(9) J. M. Johnson, *ibid.*, **134**, 459 (1940).

(10) O. K. Behrens, F. Lipmann, M. Cohn and D. Burk, *Science*, **92**, 32 (1940).

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