ethylbenzene and *m*- and *p*-xylene were separated by a procedure described previously.[9] The benzoic acid from the toluene was purified by sublimation at 100° followed by recrystallization from boiling water. Purification of the phthalic acid from *o*-xylene was made as described previously.[9]

Radiochemical Assay.—The radiochemical assay of the resulting aromatic acids and their decarboxylation products was the same as that reported previously[16] (Table IV).

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Alumina: Catalyst and Support. XXVIII.[1] Aromatization and Dehydroisomerization of 3- and 4-Methylheptane and 3- and 4-Methyl-C^{14}-heptane. Contribution to the Mechanism of Aromatization[2]

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The aromatization of 3- and 4-methylheptane along with their corresponding methyl-C^{14} compounds was investigated. The usual skeletal rearrangements observed in other such studies were also observed in the present study. The carbon-14 distribution in the aromatic compounds indicates that not only 1,2 and/or 1,3 methyl-carbon insertion reactions participate, but also 1,7 ring closure is a major contributor to the skeletal rearrangement of the methylheptanes prior to aromatization. The present study has also suggested a reasonable correlation between the various "closure" mechanisms and the catalytic sites responsible for such closures.

The studies in the preceeding papers have left little doubt that the aromatization of hydrocarbons over chromia–alumina catalysts is anything but simple. *n*-Heptane[3–5] and *n*-octane[6,7] have been shown to form cycloheptane and cyclooctane intermediates over this catalyst. The methylpentanes[8–10] and the methylhexanes[11] undergo skeletal rearrangements partially *via* 1,2 and/or 1,3 methyl-carbon insertion reactions.[12] Finally, at any particular time one or all of these reactions may be occurring. Although all these data appear to make the reaction mechanism very complex, a closer examination shows a surprising consistency from a wide variety of different compounds. For example, *n*-heptane[4,5] and *n*-octane[7] aromatize not only *via* 1,6 ring closure but aso *via* cycloheptane- and cyclooctane-type species, respectively. Apparently, closure at the terminal methyl groups is a desired path. This seems to hold true in branched hydrocarbons as well. An earlier discussion[11] on carbonaceous material formation indicated the ease of five-membered ring formation in cases where two methyl groups were separated by three methylene carbon atoms. Fulvene compounds have actually been isolated in some cases.[13] Further, the methyl-carbon insertion mechanism seems to be common with the branched hydrocarbons. That is, the aromatic products from the methylpentanes and methylhexanes can be explained by the same 1,2 and in some cases by 1,3 methyl-carbon insertion reactions, and, unifying even more, the 1,3 methyl insertion may be the closure *via* two adjacent methyl groups followed by ring cleavage. The 1,2 methyl insertion may be a 1,2 vinyl group shift.[12,14] In the present case, 3- and 4-methylheptanes were studied to show that the next higher homologous series follows this same pattern.

4-Methylheptane can cyclize *via* 1,6 closure to only *m*-xylene. If other aromatics are produced, skeletal rearrangements by possibly 1,2 methyl-carbon insertion and/or 1,7 ring closure would be necessary. 3-Methylheptane can directly yield ethylbenzene and *o*- and *p*-xylene. However, *m*-xylene is precluded. Skeletal rearrangements by either 1,2 and, in this case, 1,3 methyl-carbon insertion and/or 1,7 ring closure would allow *m*-xylene to be formed. Therefore, the formation of any aromatics other than *m*-xylene in the former case and *m*-xylene formation in the latter case would indicate skeletal rearrangements prior to aromatization.

Carbon-14 tracer studies on 3- and 4-methylheptane also appear necessary. Assuming skeletal rearrangement does occur, then the question arises as to whether it is a methyl-carbon insertion or 1,7 ring closure which is occurring. If skeletal rearrangement follows 1,7 ring closure, then the carbon-14 distribution in aromatics not produced by direct ring closure should be similar to that found in the corresponding aromatic compounds from the aromatization of methyl-C^{14}-cycloheptane.[1]

In the case of 4-methyl-C^{14}-heptane, a 1,2 methyl-carbon insertion would give *n*-octane-4-C^{14}-adsorbed species. Therefore, if this were the sole process for skeletal rearrangement, the carbon-14 distribution in the *o*-xylene isolated should be similar to that found in the *o*-xylene produced when *n*-octane-4-C^{14} was passed over this catalyst.[7] The carbon-14 distribution in the *m*-xylene would be more difficult to decipher since the possible methyl-carbon insertion reactions would leave the rearranged products labeled with carbon-14 in several different positions.

(1) For paper XXVII, see C. T. Goetschel and H. Pines, **30**, 3544 (1965).

(2) (a) Paper XVII of the series "Aromatization of Hydrocarbons"; for paper XVI, see ref. 1. (b) This research was supported by the Atomic Energy Commission Contract AT (11-1)-1196.

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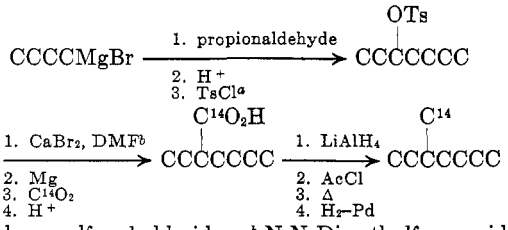
Procedure. A. 3- and 4-Methylheptane.—The commercial hydrocarbons were purified to over 99.8% purity by preparative chromatography. They were dehydrogenated at 522–527° over nonacidic chromia–alumina-B catalyst by a previously described procedure.[9] The products were analyzed by gas chromatography using an F & M Model 300 programmed-temperature gas chromatograph as described earlier.[9]

B. 3-Methyl-C^{14}-heptane.—The sequence of reactions shown in Chart I was used to synthesize this hydrocarbon in over 99% purity.

CHART I



[a] *p*-Toluenesulfonyl chloride. [b] N,N-Dimethylformamide.

C. 4-Methyl-C^{14}-heptane was prepared by a similar procedure but using *n*-propylmagnesium bromide and butyraldehyde as starting material. The dehydrogenation was carried out in the same manner as for the inactive compounds.

The reaction products from 3- and 4-methyl-C^{14}-heptane were collected in three separate cuts and the major part of each cut was selectively hydrogenated to reduce the olefins. The aromatics were separated by preparative v.p.c. From 4-methyl-C^{14}-heptane three fractions were collected: 1, containing toluene; 2, *m*-xylene; and 3, *o*-xylene. From 3-methyl-C^{14}-heptane the following fractions were collected: 1, toluene; and 3, *o*-xylene, both in pure form. The fraction 2, containing ethylbenzene and *m*- and *p*-xylene, was passed through another preparative column which separated the ethylbenzene and *m*- and *p*-xylene. Each of the samples was diluted with the corresponding aromatic compound and oxidized with alkaline potassium permanganate and the acids were purified, as described previously.[1] The specific activity of the diluted ethylbenzene was determined prior to oxidation and, therefore, the activity of the β-carbon atom on the side chain could be determined by the loss of activity when ethylbenzene was oxidized to benzoic acid. The acids were decarboxylated in boiling quinoline with copper oxide. The benzene and carbon dioxide products were analyzed for radioactivity.[1]

Discussion of Results

4-Methylheptane.—Aromatics other than *m*-xylene are produced from the aromatization of 4-methylheptane over nonacidic chromia–alumina catalyst, which demonstrates that a direct 1,6 ring closure is not the sole mechanism for aromatic formation (Table I). If it is assumed that only 1,2 methyl-carbon insertion and/or 1,7 closure are responsible for the skeletal rearrangement, then the former would give a *n*-octane species which would yield only ethylbenzene and *o*-xylene by 1,6 closure, while the latter giving methylcycloheptane would give all the aromatics.

TABLE I

DEHYDROGENATION AND DEHYDROCYCLIZATION OF 4-METHYLHEPTANE OVER CHROMIA–ALUMINA-B CATALYST AT 524° AND CONTACT TIME OF 3.0 SEC.

	—Composition, mole %[a]— Cut (length in min.)		
Conversion products	1 (4.0)	2 (5.0)	3 (6.0)
Methane	0.84	0.72	0.37
Ethane + ethylene	0.40	0.40	0.44
Propane	1.96	0.92	0.08
Propylene	1.45	1.54	1.63
Isobutane	0.09	0.09	0.06
Isobutylene	0.06	0.06	0.06
Isopentane	0.04	0.04	0.02
n-Pentane	1.73	1.83	2.03
n-Pentenes	1.77	1.65	1.77
2-Methylpentane	0.17	0.08	0.08
1,4-Pentadiene	0.13	0.09	0.03
Methylpentenes	0.65	0.44	0.44
1,3-Pentadienes	0.21	0.15	0.09
n-Heptane (enes)	0.08	0.08	0.08
n-Octane (enes)	0.05	0.05	0.04
4-Methylheptane	69.71	79.62	84.64
4-Methylheptenes	4.11	3.52	3.05
Toluene	0.78	0.64	0.37
Ethylbenzene	0.44	0.15	0.09
p-Xylene	0.81	0.42	0.22
m-Xylene	13.75	7.99	4.11
o-Xylene	1.79	0.56	0.30
Total conversion of 4-methylheptane	30.3	20.4	15.4
Aromatic conversion products	Distribution in the aromatic fraction, mole %		
Toluene	4.4	6.6	7.3
o-Xylene	10.2	5.7	5.9
m-Xylene	78.3	81.9	80.7
p-Xylene	4.6	4.3	4.3
Ethylbenzene	2.5	1.5	1.8

[a] Total carbonaceous materials: 1.73%.

The data show that *p*-xylene is produced in an even larger amount than ethylbenzene, thereby leaving doubt as to the major contribution of the 1,2 methyl-carbon insertion, followed by 1,6 ring closure. Either a 1,8 ring closure must follow the 1,2 methyl-carbon insertion and/or the 1,7 closure to a methylcycloheptane species is also occurring. The fact that the *p*-xylene formation hardly changes with time while ethylbenzene and *o*-xylene drop rapidly suggests that both *n*-octane- and methylcycloheptane-adsorbed species are being formed but that the former diminishes with time. Added support for this is the fact that *p*-xylene formation from methylcycloheptane remains nearly constant[1].

3-Methylheptane.—As to be expected, direct 1,6 closure is not the sole process occurring to yield the aromatic compounds, since a considerable amount of *m*-xylene was produced (Table II). Here, skeletal rearrangement may proceed by 1,2 and/or 1,3 methyl-carbon insertions and/or 1,7 ring closure mechanisms. The 1,2 methyl-carbon insertion would give a *n*-octane-adsorbed species, while 1,3 methyl insertion would yield a *n*-octane species and/or 3-methylheptane again. The *m*-xylene could be derived from the methylcycloheptane species produced from 1,7 ring closure or from a cyclooctane-adsorbed species.

4-Methyl-C^{14}-heptane.—The composition of the product from the reaction is given in Table III while the distribution of the radioactivity in the aromatics studied

TABLE II

DEHYDROGENATION AND DEHYDROCYCLIZATION OF 3-METHYLHEPTANE OVER CHROMIA–ALUMINA-B CATALYST AT 527° AND CONTACT TIME OF 3.0 SEC.

Conversion products	Composition, mole %[a] Cut (length in min.) 1 (5.0)	2 (5.0)	3 (5.5)
Methane	2.17	1.60	1.58
Ethane + ethylene	1.10	0.93	0.93
Propane	0.20	0.19	0.19
Propylene	0.18	0.10	0.10
n-Butane	0.97	0.91	0.76
n-Butylenes	1.60	1.47	1.46
Isopentane	0.13	0.04	0.04
Methylbutenes	0.24	0.20	0.10
1,3-Butadiene	0.05	0.04	0.04
n-Hexene	0.50	0.31	0.27
n-Hexenes	0.82	0.71	0.70
n-Heptane + olefins	0.07	0.06	0.06
n-Octane + olefins	0.08	0.06	0.06
3-Methylheptane	59.04	66.79	68.76
Methylheptenes	6.26	6.05	5.94
Benzene	0.04	0.03	0.02
Toluene	1.86	1.41	1.31
Ethylbenzene	6.31	4.99	4.74
p-Xylene	7.05	5.91	5.74
m-Xylene	3.24	1.99	1.51
o-Xylene	7.41	5.72	5.25
Styrene	0.68	0.49	0.44
Total conversion of 3-methylheptane	41.0	33.2	31.2
Aromatic conversion products	**Distribution in aromatic fraction, mole %**		
Benzene	0.2	0.1	0.1
Toluene	7.0	6.9	6.9
o-Xylene	27.9	27.8	27.6
m-Xylene	12.2	9.7	8.0
p-Xylene	26.5	28.8	30.2
Ethylbenzene	23.7	24.3	24.9
Styrene	2.5	2.4	2.3

[a] Total carbonaceous materials: 0.91%.

TABLE III

AROMATIZATION OF 3- AND 4-METHYL-C^{14}-HEPTANE OVER CHROMIA–ALUMINA-B CATALYST[a]

Aromatic conversion products	Distribution in aromatic fraction, mole % 3-Methyl-C^{14}-heptane[b] Cut (length in min.) 1 (12.0) 3.9[d]	2 (4.0) 1.4[d]	3 (7.0) 2.6[d]	4-Methyl-C^{14}-heptane[c] Cut (length in min.) 1 (5.5) 2.0[d]	2 (5.5) 2.0[d]	3 (9.0) 3.4[d]
Toluene	7.0	6.9	6.9	4.4	6.6	7.3
Ethylbenzene	23.7	24.3	24.9	2.5	1.5	1.8
o-Xylene	27.9	27.9	27.6	4.6	4.3	4.3
m-Xylene	12.2	9.7	7.9	78.3	81.9	80.8
p-Xylene	26.5	28.8	30.2	10.2	5.7	5.9
Total conversion of C_8H_{18} to aromatics	26.6	20.6	19.0	17.6	9.8	5.1

[a] The experiments were made at 522° and an hourly liquid space velocity of 1.57. The conversion to carbonaceous material was determined at the end of the experiment. [b] C_8H_{18} converted to carbonaceous material: 0.91 mole %. [c] C_8H_{18} converted to carbonaceous material: 1.77 mole %. [d] Total C_8H_{18} passed in milliliters.

is summarized in Table IV. Only the radioactivity distribution in toluene and *m*- and *o*-xylene was studied, since ethylbenzene and *p*-xylene were produced in such small quantities that isolation was impractical. The fact that the *o*-xylene has between 25 and 30% radioactivity in the ring suggests that only about 20–25% of it could be formed through a *n*-octane species, the

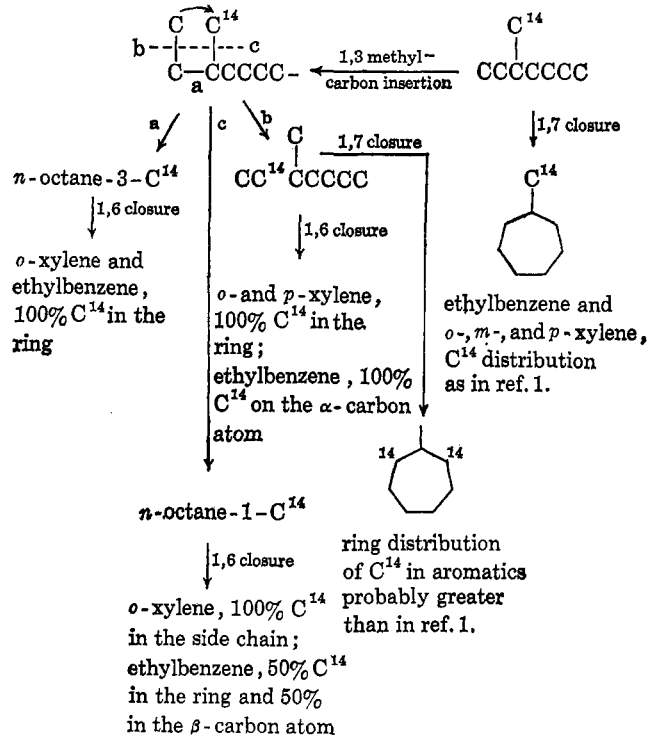
TABLE IV

RADIOACTIVITY DISTRIBUTION FROM THE AROMATIZATION OF 3- AND 4-METHYL-C^{14}-HEPTANE

Aromatic compd. (cut)	3-Methyl-C^{14}-heptane % side chain	% ring	Dif.[a]	4-Methyl-C^{14}-heptane % side chain	% ring	Dif.[a]
Toluene						
(1)	48.3	50.0	−1.7	62.0	38.5	1.5
(2)	40.3	58.0	−1.7	60.3	37.8	−1.9
(3)	40.8	58.4	−5.8	67.7	34.2	1.9
Ethylbenzene						
(1)	α, 3.7[b]	95.4[b]	−0.9[c]			
(2)	α, 2.5; β, 36.2	59.6	−1.7[c]			
(3)	α, 5.7; β, 30.9	61.9	−1.5[c]			
o-Xylene						
(1)	76.6	20.6	−2.8	67.7	29.5	−2.8
(2)	78.3	22.7	0.0	74.8	25.0	−0.2
(3)	64.4	32.5	−3.1	76.2	24.3	0.5
m-Xylene						
(1)	53.8	43.3	−2.9	88.1	14.3	2.4
(2)	45.4	56.9	2.3	90.1	12.6	2.7
(3)	47.5	52.6	0.1	88.4	11.4	−0.2
p-Xylene						
(1)	80.4	17.8	−1.8			
(2)	80.3	19.8	0.1			
(3)	79.7	19.1	−1.2			

[a] Difference between experimental value and 100% radioactivity recovery. [b] Distribution in the benzoic acid derived from ethylbenzene.

CHART II



other coming from a methylcycloheptane species.[15] The activity in the ring of *m*-xylene probably comes from this same methylcycloheptane and cyclooctane species.

The large amount of carbonaceous material associated with the aromatization of 4-methylheptane would be expected, since there are two methyl groups separated by three carbon atoms allowing the formation of cyclopentane-adsorbed species (see Discussion in ref. 11). This may explain the fact that the total aromatic formation drops rapidly with time, since the catalytic sites responsible for 1,7 ring closure may be the same

(15) Determined from the data for the radioactivity distribution in *o*-xylene from methylcycloheptane and *n*-octane-4-C^{14}.

TABLE V

RADIOCHEMICAL ASSAY DATA. DECARBOXYLATION OF ACIDS DERIVED FROM AROMATICS PRODUCED FROM 4-METHYL-C^{14}-HEPTANE

Aromatic assayed	Toluene			*o*-Xylene			*m*-Xylene		
Acid decarboxylated	Benzoic			Phthalic			Isophthalic		
Cut	1	2	3	1	2	3	1	2	3
Acid decarboxylated, mmole	0.84	0.45	0.99	0.42	0.52	0.82	0.63	0.50	0.91
Barium carbonate obtained, mmoles	0.70	0.39	0.83	0.28	0.36	0.75	1.14	0.78	1.55
Barium carbonate yield, mole %	83	87	84	35	35	46	90	78	85
Benzene obtained, mmole	0.27	0.07	0.23	0.08	0.05	0.24	0.23	0.05	0.07
Benzene yield, mole %	37	16	23	19	10	26	36	10	8
Radioactivity 10^{-3} μc./mmole									
Acid	1221	1340	1008	1264	964	1194	8630	9860	18,950
Barium carbonate	770	807	682	855[a]	721[a]	910[a]	7600	8890	16,750
Benzene	470	506	345	373	241	291	1233	1240	2,170

[a] The radioactivity of barium carbonate was multiplied by two.

TABLE VI

RADIOCHEMICAL ASSAY DATA. DECARBOXYLATION OF ACIDS DERIVED FROM AROMATICS PRODUCED FROM 3-METHYL-C^{14}-HEPTANE

Aromatic assayed	Ethylbenzene			Toluene			*o*-Xylene			*m*-Xylene			*p*-Xylene		
Acid decarboxylated	Benzoic			Benzoic			Phthalic			Isophthalic			Terephthalic		
Cut	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Acid decarboxylated, mmoles	0.73	0.46	0.29	0.94	1.20	0.74	1.37	0.98	1.10	0.79	0.29	0.81	0.97	0.62	0.89
Barium carbonate obtained, mmoles	0.64	0.36	0.29	1.03	1.21	0.75	2.12	1.19	1.37	1.54	0.42	1.56	1.90	0.64	1.73
Barium carbonate, yield, mole %	87.6	78.3	100	109	101	101	77.5	60.8	63.0	97.5	72.5	96.5	98.0	50.8	97.2
Benzene obtained, mmole	0.12	0.13	0.08	0.51	0.54	0.36	0.29	0.39	0.35	0.26	0.05	0.24	0.05	0.04	0.40
Benzene yield, mole %	16.4	28.3	27.6	54.6	45.0	48.7	21.2	39.8	31.8	32.9	17.2	29.6	5.2	6.4	45.0
Radioactivity, 10^{-3} μc./mmole															
Ethylbenzene		240	586												
Acid	1338	153	405	234	95.6	55.1	1215	279	178	897	856	116	1874	600	304
Barium carbonate	49.1	5.9	33.7	113[a]	38.3	22.4	930[b]	218[b]	115[b]	482	38.9	55.1	1508	482	242
Benzene	1277	143	363	117	55.1	29.4	250	63	58	389	48.7	61.0	333	119	58

[a] After correcting to 100% barium carbonate instead of 109%. [b] The radioactivity of the carbonate was multiplied by two.

as those causing 1,5 ring closure which would then deactivate due to carbonaceous material formation.

3-Methyl-C^{14}-heptane.—Table III and IV summarize experimental results and the radioactivity distribution in the aromatic compounds formed. It is obvious from the large amount of carbon-14 distributed in the ring of the aromatic compounds that rearrangement occurs to a large extent. As mentioned earlier in the paper, the *m*-xylene is produced probably *via* a 1,7 closure mechanism. The slight excess in the ring label of *m*-xylene from 3-methyl-C^{14}-heptane over that of methyl-C^{14}-cycloheptane may be due to a 1,3 methyl-carbon insertion reaction followed by 1,7 closure. The radioactivity distribution in the ethylbenzene and *o*- and *p*-xylene likewise can be explained as above (see Chart II). Also to be included is the possibility of a 1,2 methyl insertion giving a *n*-octane species. This would distribute the carbon-14 into the rings of *o*-xylene and ethylbenzene.

It is interesting to note that the distribution of radioactivity in the ring increases as the reaction progresses. However, the reverse is true for 4-methyl-C^{14}-heptane and methyl-C^{14}-cycloheptane. This suggests that the contribution of 1,3 methyl-carbon insertion to the aromatization of 3-methylheptane diminishes much less with time than the processes involving methylcycloheptane. This statement is a direct contradiction to much of the work done on the methylpentanes and methylhexanes. However, a close examination will reveal one major difference. In all cases when 1,3 methyl-carbon insertion was possible, 1,5 closure to cyclopentane species *via* two methyl groups was also possible. The observed decrease with time of 1,3 methyl-carbon insertions, in these cases, may be linked to the fact that the catalytic sites necessary for such insertion reactions are the same as those for 1,5 closure and, therefore, deactivate with carbonaceous material buildup. In the case of 3-methylheptane, 1,5 closure is not possible; therefore, the deactivation of the catalytic sites responsible for 1,3 methyl-carbon insertion is much less than in previous cases studied.

Conclusion.—The present investigation has revealed several possible correlations for the aromatization reaction over chromia–alumina catalysts. The main aromatization reaction proceeds *via* 1,6 ring closure. The skeletal rearrangement and the distribution of radioactivity in the aromatics can be explained by 1,2 and, in the case of 3-methylheptane, by 1,3 methyl-carbon insertion followed by 1,6, 1,7, and 1,8 ring closure and by a direct 1,7 ring closure.

Experimental Section

3-Methyl-C^{14}-heptane.—The reaction between *n*-butylmagnesium bromide (0.6 mole) and 29.0 g. (0.50 mole) of propionaldehyde gave 43 g. (74% yield) of **3-heptanol**, b.p. 90.5–91.5° (75 mm.). A solution of 29 g. (0.25 mole) of 3-heptanol in 240 ml. of dry pyridine was treated with 95 g. (0.498 mole) of *p*-toluenesulfonyl chloride according to the published procedure,[16] yielding **3-heptyl-*p*-toluenesulfonate**. The crude tosylate was treated with 120 g. (0.51 mole) of calcium bromide dihydrate in 600 ml. of N,N-dimethylformamide as described previously.[17] The yield of **3-heptyl bromide** was 28.5 g. (63.8% based on 4-heptanol), b.p. 64° (26 mm.). **2-Ethylhexanoic acid-1-C^{14}** was prepared according to the published procedure,[18] using 0.50 mole of 3-heptylmagnesium bromide and 0.036 mole of sodium carbonate containing 10 mc. of barium carbonate-C^{14}. The product was diluted with twice the volume of the inactive 2-ethylhexanoic acid. An ethereal solution of the acid was treated with 7.6 g. (0.2 mole) of lithium aluminum hydride according to a previously published procedure.[19] The ether was removed

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in vacuo and the residue, **2-ethylhexanol-1-C^{14}**, was directly acetylated with 6.0 g. (0.77 mole) of acetyl chloride and 30 ml. of pyridine by a procedure described previously.[19] The product, **2-ethylhexyl acetate-1-C^{14}**, was purified by distillation: b.p. 94.5° (21 mm.). The acetate was dissolved in about 4 ml. of 3-methylheptane and pyrolyzed at 550° as described previously,[11] yielding **3-methyl-C^{14}-heptenes**. Hydrogenation of the olefins at room temperature with 5% palladium-on-charcoal catalyst was done in a Parr hydrogenation apparatus. The 3-methyl-C^{14}-heptane weighed 6.8 g. and was over 99% pure by v.p.c. The over-all yield based on starting barium carbonate activity was 12%.

4-Methyl-C^{14}-heptane.—The reaction between *n*-propylmagnesium bromide (0.6 mole) and *n*-butyraldehyde (36 g., 0.65 mole) gave 48.4 g. (yield 83.5%) of **4-heptanol**, of over 99% purity, b.p. 72–74° (26 mm.). **4-Heptyl bromide** was made from 4-heptanol *via* the tosylate as described above for 3-heptyl bromide. The yield, starting with 29 g. (0.25 mole) of 4-heptanol, was 63.8% (28.5 g.), b.p. 64° (26 mm.). The procedure used to prepare the 4-methyl-C^{14}-heptane from 4-heptyl bromide starting with 10 mc. of barium carbonate-C^{14} was the same as described for the preparation of 3-methyl-C^{14}-heptane from 3-heptyl bromide. The hydrocarbon, which was prepared in an over-all yield of 41.6% based on the 10 mc. of barium carbonate used, was over 99% pure by v.p.c.

Catalyst.—The chromia–alumina catalyst was made according to the procedure described previously.[20] The alumina was precipitated from sodium aluminate and impregnated with chromic acid. The catalyst contained 13.8 wt. % of Cr_2O_3, its surface area was 89 m.^{2}/g., and the average pellet weight was 0.022 g.

Apparatus and Procedure.—The apparatus and procedure used were the same as those described previously.[9]

Separation of the Aromatic Hydrocarbons from the Dehydrogenation Products.—The separation of the aromatic hydrocarbons from the aromatization of 4-methyl-C^{14}-heptane was accomplished using an F & M Model 300 programmed-temperature gas chromatograph[21] with an 11 mm. × 2.5 m. preparative v.p.c. column filled with a 5% 7,8-benzoquinoline on 30–60-mesh Chromosorb. The column temperature was 75° with a helium flow of 100 cc./min. The sample recovery was the same as described previously.[7] The separation of the aromatics from 3-methyl-C^{14}-heptane was done using two separate preparative gas chromatography columns. First, a 7,8-benzoquinoline preparative column, as described above, was used to separate toluene and *o*-xylene in pure form. The ethylbenzene and *m*- and *p*-xylene, collected in one fraction, were separated using a Wilkens Autoprep gas chromatograph[22] with a $^3/_8$ in. × 20 ft. column with 5% SE-52 and 15% bentone clay on 60–80-mesh Chromosorb W. The column temperature was 80° and a helium flow of 40 cc./min. Sample injection sizes were 100 μl. The ethylbenzene was diluted with inactive ethylbenzene and the specific activity was determined. The loss in activity when the ethylbenzene is oxidized to benzoic acid corresponds to the activity on the β-carbon of the side chain.

Oxidation of the Aromatics.—After separation, the aromatics were diluted from 5 to 25 times with their corresponding inactive aromatic compounds and oxidized to their respective acids with hot alkaline potassium permanganate as described previously.[7]

Radiochemical Assay.—The radiochemical assay of the resulting aromatic acids and their decarboxylation products was the same as that reported previously[23] (Tables V and VI).

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Microbiological Hydroxylation of Saturated 17-Keto Steroids[1]

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Penicillium sp. ATCC 12,556 was previously shown to hydroxylate 3α-hydroxy-5β-androstan-17-one in the 7β position. Fermentation of 3α-hydroxy-5α-androstan-17-one with this same mold afforded the 12β-hydroxylated derivative as the principal product, whereas the 3β-OH,5α-H epimer was hydroxylated at 1α. 3β-Hydroxy-5β-androstan-17-one yielded two products, 3β,7β-dihydroxy-5β-androstan-17-one and 7β-hydroxy-5β-androstane-3,17-dione.

3α,7β-Dihydroxy-5β-androstan-17-one (7β-hydroxyetiocholanolone)[3] has recently been isolated from human urine and established as a metabolite of testosterone as well as of etiocholanolone.[4] The new metabolite was prepared by the microbiological hydroxylation of etiocholanolone with *Penicillium* sp. ATCC 12,556 and found to be identical with the urinary steroid. It was therefore of interest to study the site of hydroxylation by this organism of other saturated 17-keto steroid metabolites of testosterone, *i.e.*, androsterone, isoandrosterone, and 3β-hydroxy-5β-androstan-17-one.

Fermentation of the saturated steroids was carried out according to the procedure reported earlier.[4,5] It was expected that incubation of androsterone (I) with *Penicillium* sp. ATCC 12,556 would yield the 1α-hydroxylated derivative since Dodson and coworkers[5] have reported 1α-hydroxylation of 5α-androstane-3,17-dione and dehydroisoandrosterone with this organism. However, the principal product IIa isolated from the fermentation of androsterone did not appear to be the expected compound. Molecular rotation differences, determined for the dihydroxy ketone IIa (ΔM_D OH = −62) and its diacetate IIb (ΔM_D OAc = −107), were incompatible with 1α-hydroxyandrosterone although consistent with a 1β-hydroxy derivative.[6] Oxidation of IIa to a triketone III (Scheme I) gave a different product from that obtained by oxidation of 1α-hydroxy-5α-androstane-3,17-dione, a compound which had been prepared earlier from 5α-

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(3) The following trivial names have been used: 7β-hydroxyetiocholanolone, 3α,7β-dihydroxy-5β-androstan-17-one; etiocholanolone, 3α-hydroxy-5β-androstan-17-one; testosterone, 17β-hydroxy-Δ^4-androsten-3-one; androsterone, 3α-hydroxy-5α-androstan-17-one; isoandrosterone, 3β-hydroxy-5α-androstan-17-one; dehydroisoandrosterone, 3β-hydroxy-Δ^5-androsten-17-one.

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