REFERENCES

(1) Stahl, E., Schroeter, G., Kraft, G., and Renz, R., *Pharmazie*, 11, 633(1956).
(2) Fiori, A., and Marigo, M., *Nature*, 182, 943(1958).
(3) Nürnberg, E., *Arch. Pharm.*, 292, 610(1959).
(4) Gänshirt, H., and Malzacher, A., *ibid.*, 293, 925 (1960).
(5) Liebich, M., *Deut. Apotheker-Ztg.*, 99, 1246(1959).
(6) *Ibid.*, 100, 394(1960).
(7) Machata, G., *Mikrochim. Acta*, 1960, 79.

(8) Wollish, E. G., Schmall, M., and Hawrylyshyn, M., *Anal. Chem.*, 33, 1138(1961).
(9) Frahm, M., Gottesleben, A., and Soehring, K., *Arzneimittel-Forsch.*, 11, 1008(1961).
(10) "United States Pharmacopeia," 16th rev., Mack Printing Co., Easton, Pa., 1960.
(11) "The National Formulary," 11th ed., J. B. Lippincott Co., Philadelphia, Pa., 1960.

Composition of Gum Turpentines from Twenty-Two Species of Pines Grown in New Zealand

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Vapor-phase chromatography, following steam distillation, has provided an effective means of identifying many components of the gum turpentines of oleoresins from 22 *Pinus* species, with the probable exception of the sesquiterpenes and some trace constituents. With the improved injection instruments, it has been possible to analyze samples as small as 0.5 Gm., thus allowing the study of very low-yielding or small trees. This work confirms the specific differences previously reported for the major constituents, with two notable exceptions, *P. muricata* and *P. montezumae*. The results suggest the existence of two varieties of *P. muricata*, distinguishable by a wide difference in turpentine composition.

THE GENUS *Pinus*, as described by botanists, comprises about 100 species, several of which are divided into subspecies or varieties. The chemical composition of the gum turpentine (*i.e.*, the volatile fraction of the oleoresin obtained by wounding the sapwood) has been reported for 92 species and 2 varieties, as summarized by Mirov (1). This work, requiring oleoresin samples of the order of 500 Gm., was done in the main by fractional distillation of the turpentine, followed by determination of the densities, optical rotations, and refractive indexes of the fractions, and finally by the preparation of crystalline derivatives. It was limited by the inadequate methods for isolating and identifying many of the individual compounds which occur only in trace amounts. Physical methods such as vapor-phase chromatography and infrared spectroscopy have simplified the study of turpentine composition, and have the great advantage of being applicable to very small samples. With vapor-phase chromatography alone, however, the comparison of retention volumes depends on the availability of pure reference compounds. Purified samples of terpenes are not readily

available and their preparation is often difficult and time-consuming, especially when the compounds occur only as trace quantities. Nevertheless, the value of vapor-phase chromatography in the terpene field has already been recognized and the technique has been used by many workers (1–6).

A satisfactory vapor-phase-chromatography technique for studying the turpentine composition of *P. radiata*, *P. attenuata*, and hybrids between these two species has been developed (7). While extending this work to study individual variation in *P. radiata* (8) we decided to try the technique on a number of other species of pines grown in New Zealand. This would not only test the efficiency of the method for analyzing more complex turpentines, but would also provide a comparison between species grown in New Zealand and elsewhere.

EXPERIMENTAL

Method of Sampling.—All the oleoresin samples except one were collected from single trees growing in or near the New Zealand Forest Service Arboretum at Whakarewarewa, near Rotorua, in February 1961. The remaining sample was collected from a single tree of *P. muricata* on Huckleberry Hill, near Monterey, Calif., in 1960. Holes, 2.5 cm. in diameter, were bored into the sapwood and close-fitting tubes were inserted. With this method the oleoresin had minimum contact with the outside air, and the time required to collect the sample was usually 1 or 2 days, and in no case more than 7 days. The oleoresin samples were tightly

TABLE I.—COMPOSITION OF TURPENTINES OF OLEORESINS FROM *Pinus* SPECIES[a]

Species	Yield of Turpentine, %	n-Heptane	Uniden-tified:	n-Octane:	n-No-nane:	α-Pinene:	Cam-phene:	β-Pinene:	Myrcene:	Δ³-Carene:	α-Phel-landrene:	Limo-nene:	β-Phellan-drene:	p-Cymene:	n-Undecane:	Uniden-tified:	References
Relative retention ratios		0.14	0.23	0.29	0.64	1.00	1.21	1.54	1.78	1.94	2.08	2.48	2.63	2.94	3.12	3.88	
P. leiophylla	26.4					93.0	1.5	3.5	1.0			t					13a
P. pinea	27.9					2.5		t	2.5			95.0	1.0				18
P. jeffreyi	n.d.	86.0	t	t	1.5	t					t	t	5.5				1
P. torreyana	24.2	2.0		t	2.0	2.5		t	3.5			81.0			4.5		1
P. coulteri	17.5	1.0			3.5	44.5	1.5	4.5	16.5			3.0	16.0		8.5		10
P. coulteri[b]	17.5	t			1.5	29.5		18.0		t			49.0		9.5		1
P. sabiniana	14.2	99.0		t	1.0	t								t			1
P. massoniana	31.1					91.5	2.5	2.5	1.0			1.0	1.5				1
P. thunbergii	23.6					89.0	2.0	5.0	1.5			1.0	1.5	t			1
P. lachuensis	24.1					85.0	2.0	5.0	1.5			1.0	4.5	1.0			1
P. nigra	25.4					93.0	2.5	2.0	1.0			1.0	t				11
P. ponderosa	23.8				t	6.5	t	35.0	7.0	37.0		14.5	t	t			12–16, 22
P. palustris	18.2					65.5	1.0	31.0	1.0			1.0					1
P. elliotii	17.5					59.0	2.0	34.5	2.0			t	2.5				1
P. taeda	n.d.					87.5	2.0	3.5	3.5	t		3.5	t				17
P. montezumae	24.6					8.5	t	2.0	1.5	87.0		t	1.0	t			13a, 24, 25
P. halepensis	32.9					90.5	2.5	4.5	2.5	t		t					18
P. pinaster	24.1					90.0	1.0	6.5	1.5			1.0	t				1
P. rigida	17.8					35.5	t	42.5	2.0		t	11.0	9.0				13
P. banksiana[b]	n.d.					84.5	t	13.0		t			2.5				12
P. contorta[b]	n.d.					2.0	t	3.0		4.0		t	90.0				1
P. patula	n.d.					6.0	t	1.5	2.0	5.0	1.0	t	82.5			1.0	1
P. muricata (A)[b]	22.3					2.5		5.0		86.5		t					
P. muricata (B)	26.4					13.0	t	3.5	2.0	80.5		t				t	
P. muricata (C)	23.2					6.0		3.5	2.5	86.5		t		1.0			
P. muricata (D)	25.2					2.5		3.5	2.5	90.0		t		1.5			
P. muricata (E)	27.9					97.0	1.5	1.5	t			t		1.5			26

a Values expressed as weight per cent; t indicates < 1%.   b Samples analyzed directly after distillation at 120° and helium flow rate 21 ml./min.

stoppered and stored at about 0° whenever possible until required for analysis.

Analysis.—The oleoresin samples (20 Gm. or less) were steam-distilled at atmospheric pressure and the distillates (400 ml.) collected in ice-cooled flasks. The distillation time was about 40 minutes. The yields of the steam-volatile fractions, except from the very small samples, are given in Table I. When steam distilling the smaller oleoresin samples (*i.e.*, less than 2 Gm.) the distillate was collected in a narrow-bore syphoning tube, designed so that the steam-volatiles could be sampled directly. The turpentine samples were stored at 0° between analyses.

The steam-volatiles were chromatographed on a Perkin-Elmer vapor fractometer, model 154C, in conjunction with a Leeds and Northrup Speedomax type G recorder. Columns 1 M. in length and 4–5 mm. in diameter were packed with the following materials: 20% di-isodecylphthalate on Celite 545 (Johns-Manville); 23% squalane (May and Baker) on Celite 545; 11% squalane (May and Baker) on Celite 545; 20% silicone oil DC-200 on Celite 545; 18% polyethylene glycol (Carbowax 1500) on Chromosorb R (60–80 mesh); and 15% silicone oil (May and Baker) on Chromosorb R. The columns were tested at temperatures from 80 to 120° and with various helium flow rates. The most satisfactory overall separation of the constituents of the turpentines was obtained with a column of di-isodecylphthalate on Celite 545 at a temperature of 80° and a helium flow rate of 116 ml./min. The majority of the samples were analyzed under these conditions.

A Hamilton microliter syringe (0–10 μl.) was used to inject most samples into the gas stream. For the remaining oleoresin samples (*i.e.*, 0.5–1 Gm.) which produced only trace amounts of turpentine, a Perkin-Elmer microdipper (1 μl.) was used. The volume of steam-volatiles analyzed was varied from 0.4 to 1 μl., depending on the number of constituents present and their proportions. Each analysis was run in duplicate and the mean values reported to the nearest 0.5%. The areas under the peaks were determined with a planimeter, as some peaks were incompletely resolved, and areas of less than 1% of the sum of the peak areas were quoted as trace quantities.

Relative retention ratios using α-pinene as the reference were determined for authentic samples of known turpentine constituents with the di-isodecylphthalate/Celite column at 80°, and also with the silicone oil DC-200/Celite column at 80° as a check. The identities of the individual components of the turpentines being investigated were established by comparison with these ratios, which are given in Table II. Two minor constituents present in the turpentines have not been identified.

The turpentines were initially analyzed immediately after distillation on a di-isodecylphthalate/Celite column (1 M.) at 120° with flow rate of 21 ml./min. Limonene, β-phellandrene, *p*-cymene, and *n*-undecane peaks were not distinguishable at this temperature but were more satisfactorily resolved at a lower temperature of 80°. β-Pinene and β-myrcene were also separated at this temperature. The analyses were therefore repeated at a temperature of 80° and a flow rate of 116 ml./min. approximately 2 months after steam distillation. The

compositions (weight percentages) of the steam-volatiles determined under these conditions are listed in Table I. As the analyses of *P. banksiana*, *P. contorta*, and *P. muricata* (A) could not be repeated at 80° owing to insufficient samples, the compositions of these species determined at 120° are reported. In the case of *P. coulteri*, the compositions found at both 120 and 80° are given.

Typical chromatograms, shown in Figs. 1, 2, and 3, were obtained under the following conditions: length of column, 1 M.; internal diameter of column, 4 mm.; column packing, 20% di-isodecylphthalate on Celite 545; temperature, 80°; helium flow rate, 116 ml./min.; recorder range, 8.

DISCUSSION

In this study it has been assumed that the mole responses of the constituents to the thermal conductivity cell detector are identical and, therefore, that the area under each peak is proportional to the mole per cent of that component. Jamieson (9), however, has shown that this assumption does not hold strictly when nitrogen is used as the carrier gas, and it is probable that small differences in the mole responses of the terpenes would also be found with helium. In a further investigation we propose to purify samples of the various constituents by preparative-scale gas chromatography and to determine their mole responses using helium as the carrier gas. It is expected that the necessary correction factors will be quite small. The purified terpenes will also be used to obtain optical rotation data.

We have also assumed that isomerization, polymerization, or decomposition of the turpentine constituents did not occur on the column di-isodecylphthalate on Celite 545. The catalytic effects of the supporting materials on terpenes have been discussed by many workers and Celite has been considered to be fairly satisfactory (2, 4, 5). However, Matsuura, *et. al.* (6), preferred crushed crystallized quartz which had been previously treated with nitric acid. In the present work, samples α- and β-pinenes chromatographed on a column of silicone oil (May and Baker) on Chromosorb R gave more than one peak, suggesting that the supporting medium or liquid phase was suspect. No

TABLE II.—RELATIVE RETENTION RATIOS FOR TERPENE HYDROCARBONS AND RELATED COMPOUNDS AT 80°

Compound	Relative Retention Ratios	
	Di-isodecylphthalate	Silicone oil DC-200
n-Heptane	0.14	0.18
n-Octane	0.29	0.38
n-Nonane	0.64	0.79
α-Pinene	1.00	1.00
Camphene	1.21	1.07
β-Pinene	1.54	1.33
β-Myrcene	1.78	1.42
Δ³-Carene	1.94	1.64
α-Phellandrene	2.08	1.56
Limonene	2.48	1.87
β-Phellandrene	2.63	1.85
p-Cymene	2.94	1.74
n-Undecane	3.12	3.40

such effect was detected when the pure compounds were chromatographed on a silicone oil DC 200/ Celite 545 column.

Although it has already been shown in the hybridism studies involving *P. radiata* and *P. attenuata* (8) that the ratio of the α-pinene to β-pinene content is not affected by ultraviolet light, storage time, or by distillation, this has not been established for the other terpenes encountered in these investigations. No doubt the paraffin hydrocarbons are unaffected. α- and β-Phellandrenes are known to be rather unstable compounds and to polymerize readily. This was demonstrated by the marked decrease in the β-phellandrene content of *P. coulteri* observed when the analysis of the turpentine was repeated at 80°
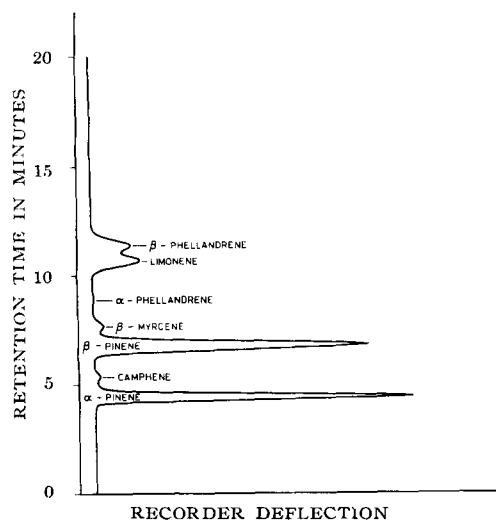

Fig. 3.—Chromatogram of turpentine from *P. muricata* (B).
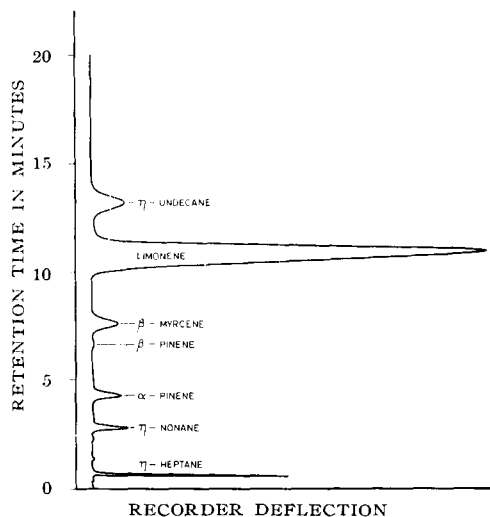
Fig. 1.—Chromatogram of turpentine from *P. torreyana.*
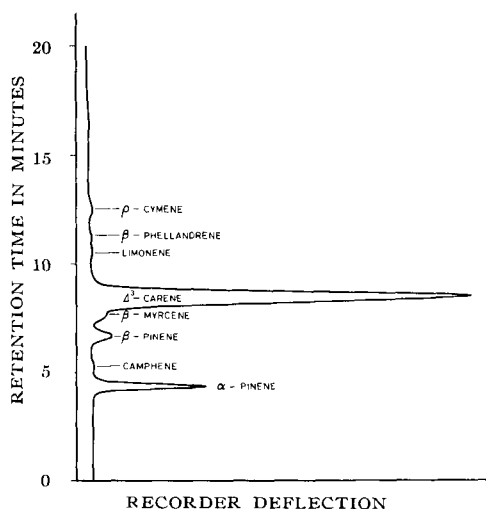

Fig. 2.—Chromatogram of turpentine from *P. rigida.*

after storage at 0° in the dark for 2 months. Hence, the composition of the volatile fraction of *P. coulteri* determined at 80° and without allowance for the polymerized phellandrene should be disregarded. Although the β-phellandrene content of *P. patula* was not affected during the same storage period, a similar decrease in the β-phellandrene content was subsequently shown. Polymerization of β-phellandrene probably also occurred during steam distillation, as suggested by Mirov (10) in his study of *P. coulteri.* He showed that polymerization was greatly decreased by distilling the turpentine under reduced pressure.

The possibility of changes in the turpentine composition during collection of the oleoresin sample cannot be overlooked. Although precautions were taken during the sampling operation, these were probably not entirely adequate in preventing some changes in the less stable turpentine constituents.

An unidentified compound in concentration from 1 to 7% (relative retention ratio 3.00 at 120°) was detected in the turpentines of *P. coulteri, P. ponderosa, P. contorta, P. patula, P. montezumae,* and *P. muricata* (samples A, B, C, D) when chromatographed immediately after distillation at 120°. This compound was apparently degraded on standing.

In view of these considerations the compositions of the turpentines given in Table I cannot be assumed to be identical with those in the living trees.

A comparison of the results given in Table I with those already published suggests that high-boiling constituents, such as the sesquiterpenes, were not detected. While we have reduced the possibility of changes in the turpentine caused through prolonged steam distillation, it is probable that the high-boiling constituents have remained in the rosin fraction of the oleoresin. Distillation of the oleoresin under reduced pressure is necessary to isolate all the volatile components (1). On the other hand, it is possible that in chromatographing the turpentines at the low temperature of 80°, the high-boiling constituents have remained on the column.

β-Myrcene appears to be more widely distributed in the genus *Pinus* than was hitherto thought. Mirov (1) notes that its presence has been recorded in eight species including *P. nigra* (11), *P. ponderosa* (12–16), *P. taeda* (17), and *P. halepensis* (18). While *P. coulteri* was found to contain 18% β-pinene when chromatographed at 120° (Table I), the analysis of this sample at 80°, even though it is not entirely reliable, suggests that probably the greater proportion of this 18% would have been myrcene. The ease of polymerization of β-myrcene noted by McGimpsey and Murray (19) in the study of *Podocarpus spicatus* (matai) could perhaps account for the fact that myrcene has seldom been found in pines.

The occurrence of β-phellandrene and camphene in small amounts is also more widespread than was supposed (1). α-Pinene, not detected previously in *P. pinea* and *P. torreyana*, has been found in the New Zealand specimens of these species.

We have tentatively identified *p*-cymene in *P. sabiniana, P. luchuensis, P. ponderosa, P. montezumae, P. thunbergii,* and *P. muricata* (samples B, C, D). This compound has previously been reported only in *P. sylvestris* (20) and *P. taeda* (11). α-Phellandrene has also been tentatively identified in *P. patula, P. rigida,* and *P. jeffreyi.*

Two or more of the paraffin hydrocarbons *n*-octane, *n*-nonane, and *n*-undecane have been tentatively identified in each of the turpentines containing *n*-heptane, *P. jeffreyi, P. torreyana, P. coulteri,* and *P. sabiniana.*

Refinements in the experimental procedure such as the use of capillary columns, the extremely sensitive ionization detectors, temperature programming, or preliminary separation and concentration of fractions of the turpentines, could establish the existence of further components.

Botanical Aspects

In comparing the results with those summarized by Mirov (1) we find, in general, a close agreement, at least for the major constituents. This was to be expected, because Mirov (1, 21) has found that there is little variation throughout the growing season within the individual tree, and that apparently the composition of the turpentine is affected little, if at all, by variations in the habitat. He has concluded that composition of turpentine is a stable, "genetically fixed character."

On the other hand, it was also to be expected that our results would differ, in some ways, from those already published. Some differences may have resulted from variation in the methods of collection and analysis. Others may have arisen from genetic variation between groups of trees, or between individual trees, within a species. Little is known about variation of turpentine composition within species, but the evidence (1) suggests that some species are nearly uniform (*e.g., P. merkusii*), whereas others are very heterogeneous (*e.g., P. ponderosa*). There is, therefore, quite a high probability that our results, most of which represent only one individual tree per species, would include some which deviate markedly from the values reported previously. In particular, we wish to discuss three species which evidently vary widely in turpentine composition.

P. ponderosa.—This species has a wide geographic distribution, and has become genetically dif-

ferentiated into a number of morphological and physiological forms. Analyses by Mirov and co-workers (12–16) of turpentines from 12 widely separate localities within its natural range, and another analysis by Goldblatt and Burgdahl (22) have revealed consistently high percentages of Δ³-carene and, generally, of β-pinene. But there is a wide variation, both in the proportions of the major constituents and in the occurrence and percentages of the minor constituents. Our result falls well within this range of variation, and, in so far as it is comparable with Mirov's data, it suggests a close kinship between the New Zealand tree and the *P. ponderosa* of southwest Idaho or California.

P. montezumae.—This name is applied to a complex assortment of populations, most of which are found in Mexico. Martinez (23) has formally divided the complex into two varieties and one form. Two independent analyses by Iriarte (24) and Mirov (13a) of samples collected near Uruapan, Michoacan, agreed in finding α-pinene to constitute 96–98% of the turpentine. For a sample from Chiapas, Iloff and Mirov (25) reported the composition: 72% α-pinene, 7% limonene, 8% heptane, and 8% longifolene. Mirov (1) has concluded that these results may represent two distinct varieties, or two distinct species, or possibly one species in Michoacan and a swarm of interspecific hybrids in Chiapas.

Our results might suggest that the New Zealand sample came from *P. ponderosa* rather than from *P. montezumae,* but this seems unlikely. In its morphological characters, the tree in question is much more like *P. pseudostrobus* than *P. ponderosa,* and it might well be *P. montezumae* var. *lindleyi* Loudon. Further work on the turpentine composition of *P. montezumae* and related species is needed.

P. muricata.—Our sample A of this species contained about 86% of Δ³-carene (Table I). It was collected on Huckleberry Hill, near Monterey, Calif. Mirov (26) had previously analyzed a sample collected near Fort Bragg, about 350 km. north of Monterey, and found the turpentine to consist almost entirely of α-pinene (98–99%). Some unpublished work by Mirov indicated that the turpentine of the Fort Bragg population might differ considerably in its optical rotation from that of the Huckleberry Hill population (27). Our result for the Huckleberry Hill sample was open to various interpretations, and as a check we sampled four trees growing near Rotorua.

Three of these belonged to a group which, from their circumstances and morphological characters, were judged to be very closely related to one another. The uniformity of their turpentines supports this judgment (Table 1, samples B, C, and D). The fourth tree (sample E) was an isolated specimen, some 8 km. away. It differed considerably in morphological character from the others and yielded turpentine essentially the same as that of the Fort Bragg trees sampled by Mirov (26). Although only superficial observations have been made on the morphology of these four trees and of *P. muricata* elsewhere in New Zealand, we suggest that the data may represent a taxonomic discontinuity in *P. muricata,* two groups being distinguishable as follows: (*a*) somewhat squat, bushy trees with hemispherical crowns when grown in the open; dense green foliage; extremely spiny cones; and turpen-

tine predominantly of Δ^3-carene; (*b*) generally taller trees with conical crowns; foliage distinctly bluish, the needles thicker than in (*a*); cones with the apophyses more rounded on the outer side and generally less spiny than in (*a*); and turpentine predominantly α-pinene.

Unfortunately, the lineage of the New Zealand specimens cannot be traced back to a known provenance in California. Nevertheless, the scheme suggested not only fits the chemical facts so far, but also agrees closely with the morphological work of Duffield (27). His conclusion was that the mainland populations of *P. muricata* could well be divided into two varieties, one of which, including the Huckleberry Hill population and the typical form near San Luis Obispo, stretched from the Annapolis area southward, while the other, including the Fort Bragg population, stretched from Annapolis northward.

Further close collaboration between botanists and chemists studying this species is highly desirable; particularly useful would be a detailed population analysis in the Annapolis area where, according to Duffield (27), the trees show a morphological transition between the typical variety and the northern one.

REFERENCES

(1) Mirov, N. T., *U. S. Dept. Agr. Forest Serv. Bull.*, No. 1239(1961).
(2) Liberti, A., and Cartoni, G. P., "Gas Chroma-tography," Butterworths Scientific Publications, London, 1958, p. 321.
(3) Cvrkal, H., and Janák, J., *Collection Czech. Chem. Communs.*, 24, 1967(1959).
(4) Groth, A. B., *Svensk Papperstidn.*, 61, 311(1958).
(5) Zubyk, W. J., and Conner, A. Z., *Anal. Chem.*, 32, 912(1960).
(6) Matsuura, T., Komae, H., Aratani, T., and Hayashi, S., *J. Chem. Soc. Japan*, 64, 791(1961); (*a*) *ibid.*, 64, 795, 799(1961).
(7) Bannister, M. H., Brewerton, H. V., and McDonald, I. R. C., *Svensk Papperstidn.*, 62, 567(1959).
(8) Bannister, M. H., Williams, A. L., McDonald, I. R. C., and Forde, M. B., unpublished.
(9) Jamieson, G. R., *J. Chromatography*, 3, 464(1960).
(10) Mirov, N. T., *Ind. Eng. Chem.*, 38, 405(1946).
(11) Bardyshev, I. I., and Bardysheva, K., *Zhur. Priklad. Khim.*, 25, 1231(1952); through *Chem. Abstr.*, 47, 2507c (1953).
(12) Haagen-Smit, A. J., Redemann, C. T., Wang, T. H., and Mirov, N. T., THIS JOURNAL, 39, 260(1950).
(13) Mirov, N. T., *ibid.*, 40, 410(1951); (*a*), *ibid.*, 40, 550(1951).
(14) Iloff, P. M., and Mirov, N. T., *ibid.*, 43, 373(1954).
(15) Mirov, N. T., and Iloff, P. M., *ibid.*, 45, 153(1956).
(16) Mirov, N. T., and Iloff, P. M., *ibid.*, 47, 404(1958).
(17) Mirov, N. T., Wang, T. H., and Haagen-Smit, A. J., *ibid.*, 38, 403(1949).
(18) Mirov, N. T., and Iloff, P. M., *ibid.*, 44, 186(1955).
(19) McGimpsey, J. R., and Murray, J., *J. Appl. Chem.*, 10, 340(1960).
(20) Cameron, D. W., and Sutherland, M. D., *Perfumery Essent. Oil Record*, 50, 200(1959).
(21) Mirov, N. T., "The Physiology of Forest Trees," Ronald Press, New York, N. Y., 1958, p. 251.
(22) Goldblatt, L. A., and Burgdahl, A. C., *Ind. Eng. Chem.*, 44, 1634(1952).
(23) Martinez, M., "Los Pinos Mexicanos," 2nd ed., Ediciones Botas, Mexico, 1948.
(24) Iriarte, J., *Quimica Mex.*, 4, 117(1946).
(25) Iloff, P. M., and Mirov, N. T., THIS JOURNAL, 42, 464(1953).
(26) Mirov, N. T., *J. Forestry*, 45, 659(1947).
(27) Duffield, J. W., Ph.D. thesis, University of California, Berkeley, 1951.

Amino Ketones: Kinetics of *In Vitro* Antibacterial Activity

By SHU-SING CHENG†, SIGURDUR JONSSON‡, and FRED T. SEMENIUK

α- and β-Amino ketone analogs of amino acids were tested *in vitro* against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus anthracis, Saccharomyces cerevisiae,* and *Candida* spp. by serial broth dilution and agar diffusion methods and found to be active as growth-inhibitors at concentration levels which varied with the test culture. The study of the growth curve of a test bacterium in a liquid medium with and without the presence of the drug provides an accurate means for the *in vitro* evaluation of the antibacterial activity of potential chemotherapeutic agents. It serves also as a means for studying the dynamics as well as the mode of antibacterial action. Since the generation time and the numbers of generations of microbial growth occur before stationary phase can be calculated, the inhibitory activity of different drugs may be compared on this basis.

THE growth-inhibitory activity of β-amino ketone analogs of β-alanine against *Escherichia coli* and *Staphylococcus aureus* has been reported recently (1). This leads to speculation that α-amino ketone analogs of α-amino acids might possess similar activity. This study was then undertaken in order to investigate the potential antimicrobial action of these two categories of amino ketone analogs of amino acids.

PROCEDURES AND RESULTS

There are three general methods for determining drug sensitivity of bacteria currently in use: the serial broth dilution, agar diffusion, and agar plate dilution methods. For the *in vitro* evaluation of antimicrobial activity of amino ketone hydrochlorides, the first two methods were selected.

Agar Diffusion Method.—*Staphylococcus aureus, Bacillus anthracis,* and *Pseudomonas aeruginosa* were used as test organisms. Stock culture was maintained on slants of peptone-casein agar and transferred to fresh slants about once a week. The assay plates and inocula were prepared according