DEGRADATION OF ISOPRENOID COMPOUNDS BY MICROORGANISMS

I. Isolation and Characterization of an Isoprenoid-Degrading Bacterium, *Pseudomonas citronellolis* n. sp.[1]

WERNER SEUBERT[2]

Section on Enzymes, National Heart Institute, Bethesda, Maryland

Received for publication August 10, 1959

In recent years, remarkable progress has been made in elucidating the biosynthetic pathways of isoprenoid compounds (Gould, 1958; Lynen *et al.*, 1958). The degradation of these compounds, however, has not been studied; hence an investigation of the bacterial degradation of citronellol and farnesol was undertaken. A study of the catabolism of these compounds, containing only two and three isoprenoid units respectively, could reveal some basic reactions of isoprenoid degradation in general.

This paper is concerned with the isolation and characterization of a citronellol- and farnesol-oxidizing bacterium isolated from soil by the enrichment culture technique. In addition, some of the over-all chemical reactions carried out by cell suspensions and dried cell preparations from the organism are reported.

MATERIALS AND METHODS

Isolation of the organism. The organism was isolated from soil collected under pine trees in northern Virginia. Approximately 10 g of soil were inoculated into 30 ml of a medium of the following composition: citronellol, 180 mg; K_2HPO_4, 630 mg; KH_2PO_4, 182 mg; NH_4NO_3, 100 mg; $MgSO_4 \cdot 7H_2O$, 20 mg; $CaCl_2 \cdot 2H_2O$, 10 mg; $FeSO_4 \cdot 7H_2O$, 10 mg; $Na_2MoO_4 \cdot 2H_2O$, 0.06 mg; $MnSO_4$, 0.06 mg; water, 100 ml. Citronellol and ammonium nitrate were the sole carbon and nitrogen sources. No additional factors were found to be required for growth.

Cultures were incubated aerobically at 30 C. After two successive subcultures in the liquid medium using 10 per cent inocula, the organism was isolated as a pure culture by the streak-plate technique. It can be stored on agar slants at 0 C for at least 6 months.

[1] A culture of this organism has been deposited in the American Type Culture Collection.

[2] Present address: Max-Planck-Institut für Zellchemie, München, Germany.

Large scale culture of the organism. For the production of cells in quantities sufficient for cell suspension studies, the organism was cultivated in 15-L volumes in 20-L carboys. The growth medium contained 3.0 g of citronellol per L (solubility, 80 mg per L), but otherwise it was identical with the enrichment culture medium. Cultures were aerated with sterile air at 30 C, and, after the odor of citronellol had disappeared (3 to 4 days), the cells were harvested in a Sharples centrifuge. Harvesting at an earlier stage usually resulted in inactivation of the cells caused by toxicity of the excess citronellol concentrated with the cells during centrifugation. Under these conditions, the average yield was 50 to 60 g wet weight per carboy. The cell paste was washed two or three times with cold water and either stored at 0 C for fresh cell suspension studies, or lyophylized and stored at −10 C.

Identification procedures. The *Manual of Microbiological Methods* of the Society of American Bacteriologists (1957) was used for the Kopeloff and Beerman modification of the Gram stain, the Conn and Fisher modification of Baily's flagella stain, Tittsler's method for nitrate determination, and Kovac's formula for the indole reagent. All additional media used in the identification of the organism were prepared as described by Stitt *et al.* (1948).

Assay for citronellol. The method employed for the quantitative determination of citronellol may be summarized as follows:

$$R{-}CH_2OH + O_2 \xrightarrow{CrO_3} R{-}COOH + H_2O$$

$$R{-}COOH + Cl{-}COOC_2H_5 + N(C_2H_5)_3 \rightarrow R{-}CO{-}O{-}COOC_2H_5 + N(C_2H_5)_3 \cdot HCl$$

$$R{-}CO{-}O{-}COOC_2H_5 + NH_2OH \rightarrow R{-}CONHOH + CO_2 + C_2H_5OH.$$

To 1 to 5 μmoles citronellol in 2 ml water, 2 ml of 0.05 N sodium dichromate in 10 N sulfuric acid are added. After standing for 3 hr at room

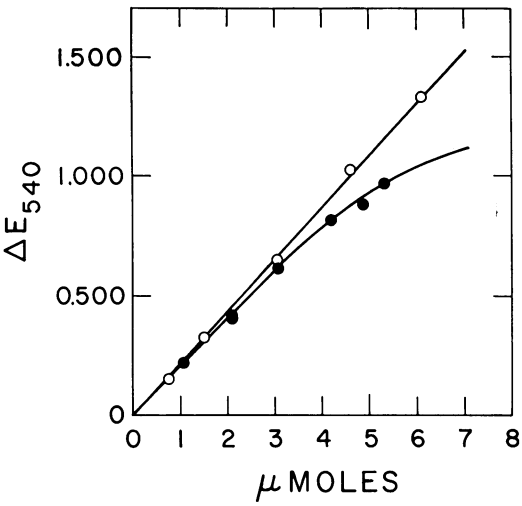


Figure 1. Quantitative determination of citronellol after conversion to hydroxamate: ●, citronellol; ○, citronellic acid. The acid was extracted into ether from aqueous solution and converted to the hydroxamate, as described in the text for citronellol. Final volume, 2 ml; path length, 1 cm.

temperature, the mixture is extracted three times with 3 ml ether, and the combined ether extracts are washed twice with 2 ml water to remove small amounts of chromic acid. The ether extract is dried with anhydrous sodium sulfate for 1 hr and concentrated to a volume of 1 to 2 ml. To form the mixed anhydride of the acid, 0.01 ml triethylamine and 0.01 ml ethylchloroformate are added at 0 C. After 20 min, 0.5 ml of a solution containing equal volumes of ethanol and 2 M aqueous hydroxylamine hydrochloride, pH 7.5, is added to form the hydroxamate. The solvent is evaporated by directing a rapid stream of N_2 over the surface of the solution, and the residue is dissolved in 0.5 ml water. After addition of 1.5 ml of ferric chloride reagent (Lipmann and Tuttle, 1945), the absorption of the colored ferric-hydroxamate complex is determined at 540 mμ. The molar absorption coefficient for citronellol, ϵ_{544}, is 4×10^2 cm²/mole. For the calculation of this figure, the experimental values were corrected by subtracting a blank value, obtained by treating a sample without citronellol in a similar fashion.

In figure 1 are shown the results of this procedure in the assay of equimolar amounts of citronellol and chemically synthesized citronellic acid. The lower values obtained with citronellol can be explained by decomposition during the oxidation with chromic acid. However, since there is a linear relationship between the amounts of citronellol added and hydroxamate formed in the range of 0 to 4 μmoles, the method is valid for the quantitative determination of citronellol in this range.

Other analytical methods. Oxidation of substrates with cell suspension was followed by measuring oxygen consumption in the Warburg apparatus. Carbon dioxide was absorbed in 2 N KOH and determined manometrically after liberation with 2 N sulfuric acid. Absorption spectra were determined with the Cary recording spectrophotometer, and fluorescence spectra with the Aminco Bowman spectrofluorometer; all other optical measurements were done with the Beckmann spectrophotometer. Radioactivity was assayed with a Tracerlab automatic flow counter; all data were corrected for self-absorption.

Chromatography of alcohol extracts of cells incubated with 2-C^{14} acetate. A column of aluminum oxide was prepared by mixing 620 g of aluminum oxide with about 700 ml of chloroform and transferring the suspension to a glass column. After the aluminum oxide had settled it was washed with 500 ml *n*-butanol. The final column dimensions were 22 cm (length) by 7 cm (diameter). The alcoholic extract (see Results and Discussion) was concentrated by evaporation of the solvent, and the residue, a brown oil, was dissolved in 30 ml of *n*-butanol. Any insoluble material was discarded after centrifugation. The soluble portion was added to the column, and the first pigment was eluted with 700 ml of *n*-butanol (fraction I). A yellow band remaining at the top was then eluted with water-saturated *n*-butanol (fraction II). Each of the eluates was dried by evaporation of the solvent.

Materials. Citronellol, citronellal, and DL-camphoric acid were purchased from the Aldrich Chemical Company; squalene, from Mann Research Laboratories; farnesol, from K and K Laboratories, Inc.; isovaleric acid, from Fischer Scientific Company; citraconic acid, itaconic acid, and β-hydroxy-β-methylglutaric acid, from the California Foundation for Biochemical Research; and 2-C^{14}-acetate from the Volk Radiochemical Company.

Citronellic and geranic acids were prepared by

oxidation of the corresponding aldehydes (Semmler, 1891). Dimethylacrylic acid was synthesized from mesityloxide (Smith *et al.*, 1955).

RESULTS AND DISCUSSION

Morphological characteristics of the organism. The organism, measuring 0.5 by 1.0 to 1.5 μ, is a gram-negative rod, which usually occurs singly but occasionally in pairs. It possesses a single polar flagellum.

Surface colonies observed on the basal medium containing 2 per cent agar are transparent, pinpoint, raised circular colonies with slightly wrinkled margins. Agar slants showed moderate, spreading growth, whereas on potato slants there was glistening, spreading growth with a brownish color.

Nutritional characteristics. Various substances were examined in concentrations of 0.2 per cent for their ability to replace citronellol in the basal medium as single substrate for supporting growth. Of these, glucose, acetate, farnesol, and ionone were each found to support growth, but no growth was observed on squalene or camphoric acid. In similar growth studies potassium nitrate, ammonium sulfate, peptone (Difco), and yeast extract (Difco) were each tested singly at a concentration of 0.1 per cent as replacements for ammonium nitrate, and each was found to be a suitable nitrogen source.

Growth occurs anaerobically only in the presence of nitrate. Growth is vigorous over the range of 25 to 37 C, with optimal growth at 31 C.

Standard microbiological tests were performed with the following results:

Action on carbohydrate in phenol red media (Difco), Durham tubes in place: acid but no gas from glycerol; neither acid nor gas from lactose, galactose, maltose, glucose, dulcitol, inositol, mannitol, inulin, dextrin, sucrose, arabinose, and levulose.

Gelatin stab in horsemeat infusion broth with 12.5 per cent gelatin: growth but no liquefaction.

Broth: turbidity developed within 24 hr with ring and white sediment.

Litmus milk: became alkaline; no coagulation.

Defibrinated rabbit blood agar slants: growth and hemolysis.

Citrate agar: supports growth.

Methyl red test: negative.

Acetylmethylcarbinol: not produced.

Nitrates reduced to nitrites in the basal medium.

Hydrogen sulfide not produced in a medium containing 2 per cent proteose peptone, 1.5 per cent agar, 0.05 per cent lead acetate, and 0.1 per cent glucose.

Indole not produced in tryptophan broth.

Classification of the organism. By virtue of the characteristics listed above the organism is a member of the genus *Pseudomonas.* It does not correspond to any of the species listed in *Bergey's Manual of Determinative Bacteriology* (Breed *et al.*, 1957). It is therefore concluded that this is a hitherto undescribed species and the name *Pseudomonas citronellolis* n. sp. is proposed. The specific epithet is the genitive singular of citronellol.

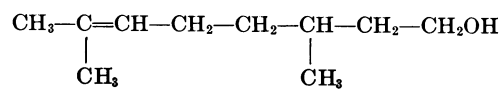
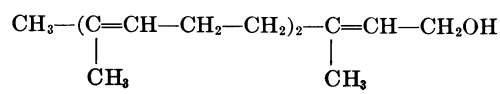
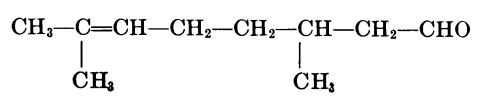
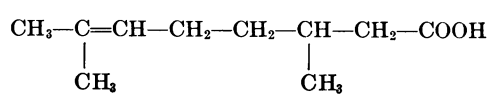
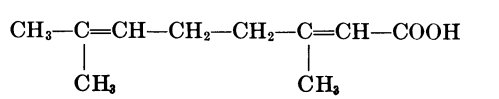
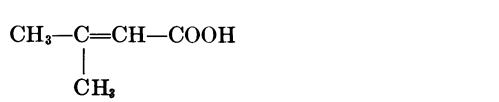
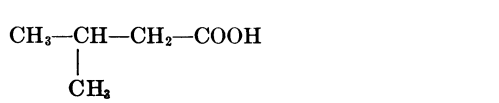
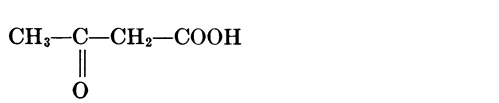
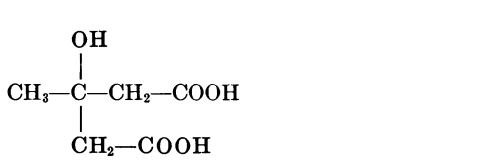
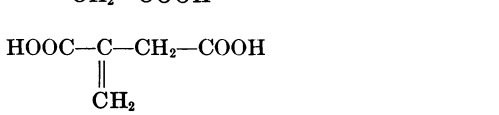
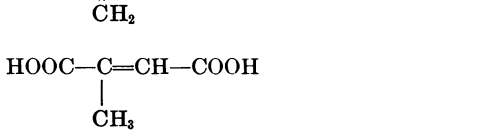
Oxidation of citronellol and related compounds by suspensions of dried and intact cells. To obtain information about the mechanism of degradation of citronellol and farnesol, the oxidation of these and a number of related compounds by suspensions of lyophylized and intact cells was studied in the Warburg apparatus. These compounds included citronellal, citronellic acid, and geranic acid, which would correspond respectively to the reaction products of two successive oxidative attacks on the alcoholic group of citronellol and oxidation on the α,β-bond of the carbon chain. Dimethylacrylic acid could be formed by oxidative cleavage at the δ-position, and reduction of the double bond could then produce isovaleric acid. Both of these latter acids could be converted to acetic acid *via* β-hydroxy-β-methylglutaric acid and acetoacetic acid. The presence of this reaction sequence in mammalian and bacterial systems has already been demonstrated (Bachhawat *et al.*, 1956; Knappe and Lynen, 1958). Itaconic and citraconic acids could be formed by oxidation at the γ-position.

As shown in table 1, all compounds tested were oxidized with the exception of β-hydroxy-β-methylglutaric acid, itaconic acid, and citraconic acid. The failure of these compounds to be oxidized does not exclude them as possible intermediates, since the enzymes necessary for converting the free acid to an activated form might be missing. Such has been found to be the case with intermediates in fatty acid oxidation (Stadtman and Barker, 1949).

Balance studies with citronellol, isovalerate, and acetate revealed that these compounds were

TABLE 1

*Oxidation of citronellol and related compounds by cell suspensions**

Substrate		O_2 Uptake
		μmoles/hr
Citronellol		13.0†
Farnesol‡		3.6
Citronellal		7.0
Citronellic acid‡		6.3
Geranic acid‡		5.4
Dimethylacrylic acid‡		2.3
Isovaleric acid		6.7
Acetoacetic acid		3.5
Acetic acid		13.4
β-Hydroxy-β-methylglutaric acid		0
Itaconic acid		0
Citraconic acid		0

* Reaction mixtures, containing 1 to 5 μmoles of substrate, 160 μmoles potassium phosphate buffer pH 7.2, and 10 mg dried cells in a final volume of 1.8 ml, were incubated at 27 C under air in the Warburg apparatus.

† These values were corrected for endogenous oxygen uptake by substracting the value for a control without substrate.

‡ In these experiments dried cells were replaced by 100 mg (wet weight) of washed resting cells.

not completely oxidized. Typical results with acetate as substrate are recorded in figure 2. In table 2 are summarized the data for oxygen uptake and carbon dioxide production at various concentrations of each of the three substrates. These data are consistent with the following equations:

Citronellol:

$$C_{10}H_{20}O + 8\tfrac{1}{2}O_2 \rightarrow (CH_2O)_6 + 4\ CO_2 + 4\ H_2O$$

Isovaleric acid:

$$C_5H_{10}O_2 + 3\tfrac{1}{2}O_2 \rightarrow (CH_2O)_3 + 2\ CO_2 + 2\ H_2O$$

Acetic acid:

$$C_2H_4O_2 + O_2 \rightarrow CH_2O + CO_2 + H_2O.$$

Thus each of the three substrates is converted to carbon dioxide, water, and an unknown product; the latter is of the same oxidation level in all three cases.

Acetate metabolism in dried cells. Although there is no direct experimental evidence to show that citronellol or isovalerate is converted to acetate in this organism it seems likely that acetate might be an intermediate in the degradation of these compounds; it further seems likely that the incompletely oxidized end products from all three of these substrates might be identical. Such an assumption is favored by the results of studies on isovalerate degradation, which revealed acetate to be an intermediate (Bachhawat *et al.*, 1956; Lynen *et al.*, 1959). Since C^{14}-labeled acetate is readily available, this compound was chosen for the further investigation of the nature of the incompletely oxidized products.

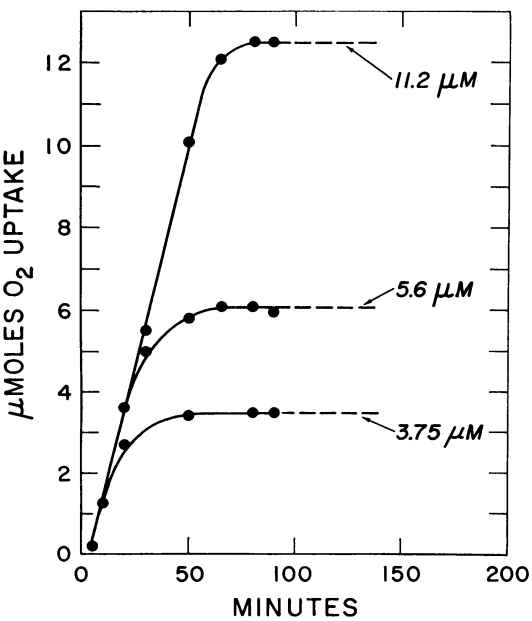


Figure 2. Effect of acetate concentration on the rate and extent of oxygen uptake by dried cells. Conditions as described in table 1 for acetate.

TABLE 2

*Balance studies with acetate, isovalerate, and citronellol**

Substrate		Total O_2 Uptake	Total CO_2 Liberation	Ratio: μMoles O_2 to μMoles Substrate	Ratio: μMoles CO_2 to μMoles substrate
	μmoles	*μmoles*†	*μmoles*†		
Acetate	3.75	3.5	4.0	0.94	1.07
	5.6	6.1	5.8	1.09	1.09
	11.2	12.6	12.9	1.12	1.14
Isovalerate	0.72	2.5	1.4	3.48	1.96
	2.16	7.2	3.4	3.35	1.6
	3.6	12.6	6.9	3.5	1.93
Citronellol	0.84	7.3	4.0	8.7	4.7
	1.68	14.5	7.5	8.6	4.45

* Reaction mixtures contained substrates as indicated, 160 μmoles potassium phosphate buffer, pH 7.2, and 10 mg dried cells in a final volume of 1.8 ml. Incubations were carried out at 27 C in double side arm Warburg vessels. When the rate of oxygen uptake had reached that of the endogenous controls the reaction was stopped.

† All values are corrected for endogenous respiration.

When 2-C^{14}-labeled acetate was incubated with dried cells and samples of the reaction mixture assayed for radioactivity after drying at acid or alkaline pH, it was determined that the radioactive products were not volatile under these conditions. About 50 to 60 per cent of the radioactivity remained with the cellular residue after repeated extractions with water.

Upon extraction with absolute alcohol a yellow fluorescent substance was obtained as well as about 10 per cent of the residual radioactivity. To obtain more of this material for chemical studies up to 20 g of acetate were incubated with dried cells for a longer period. The possibility that under these conditions the fluorescent material could have been synthesized by a contaminant is unlikely, since the resinous product obtained had the same spectral characteristics as the product isolated from 15 L of growth filtrate,

TABLE 3

*Rate of acetate utilization and end product formation**

Incubation Period	Dried Alkaline	Dried Acid
days	*cpm*	*cpm*
0	2.8×10^6	about 2.0×10^4
1	2.25×10^6	2.2×10^5
2	2.0×10^6	1.0×10^6
3	1.7×10^6	1.4×10^6
4	1.55×10^6	1.4×10^6
5	1.35×10^6	1.38×10^6

* The reaction mixture contained 1 L of 0.1 M potassium phosphate buffer, pH 7.2, 10 g potassium acetate containing 10 μc 2-C^{14}-acetate, and 5 g dried cells. Incubation temperature was 25 C, and the pH was maintained at 7.2 throughout the incubation by addition of 2 N sulfuric acid. To avoid accumulation of $C^{14}O_2$ formed during the incubation, the mixture was continually aerated.

which had been aerated under sterile conditions. Subsequent studies of the pigments isolated from both sources (from growth filtrates or from dried cell incubation mixtures) revealed that they behaved in identical fashion when chromatographed on paper and on aluminum oxide.

The results from one of these large scale experiments with dried cells and labeled acetate are summarized in table 3. Since the end products are not volatile under acid conditions, but acetic acid is, their rate of formation could be followed by measuring radioactivity of samples of solution dried at pH 2 to 3. The rate of acetate oxidation to carbon dioxide, on the other hand, could be ascertained by measuring radioactivity of samples dried at pH 8 to 10. The decrease in radioactivity of the alkaline-dried samples (table 3, column 2) indicates that approximately 50 per cent of the acetate was converted to carbon dioxide. Complete utilization of acetate is indicated when both acid- and alkaline-dried samples give identical counts (4 to 5 days).

After 5 days the reaction was stopped by heating for 1 min at 100 C. After acidification to pH 2 the cellular debris was removed by centrifugation and washed twice with water. The residue was extracted with absolute alcohol until no further yellow pigment was extracted. The insoluble residue was dried.

This type of experiment was repeated several times with quantities of acetate ranging from 5 to 20 g, and the distribution of the radioactivity in the various fractions determined. The following average values were obtained: aqueous filtrate plus water wash, 35 to 45 per cent; alcohol extract, 8 to 11 per cent, water- and alcohol-insoluble residue, 45 to 55 per cent.

As shown below, most of the radioactivity of the alcoholic extracts was contained in two fluorescent pigments, whereas that in the insoluble residue was in the form of protein-bound amino acids, suggesting that the dried cells are still capable of protein synthesis. Attempts to inhibit protein synthesis by adding chloromycetin to the incubation mixtures (20 μg per ml), and thus shift the distribution of radioactivity in favor of the alcohol soluble pigments, were not successful.

Isolation of two fluorescent pigments from the alcohol extracts. When the alcohol extract described above was analyzed chromatographically on Whatman no. 1 filter paper with water-saturated amyl alcohol as solvent, two fluorescent spots with R_f values of 0.42 and 0.81 appeared. Only the more rapidly moving spot was radioactive, and no other radioactive spots could be detected.

Chromatography on aluminum oxide (see Materials and Methods) resulted in separation of the alcoholic extract into two radioactive yellow fractions: fraction I contained 44 per

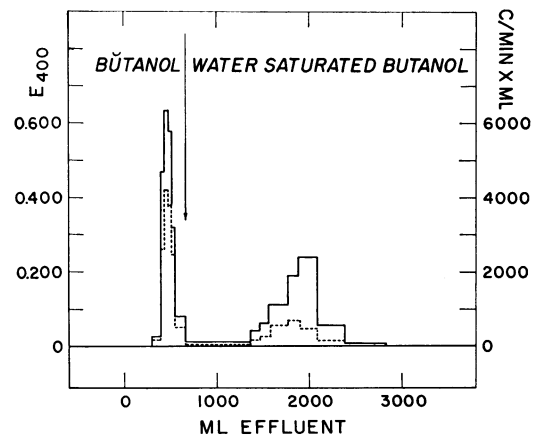
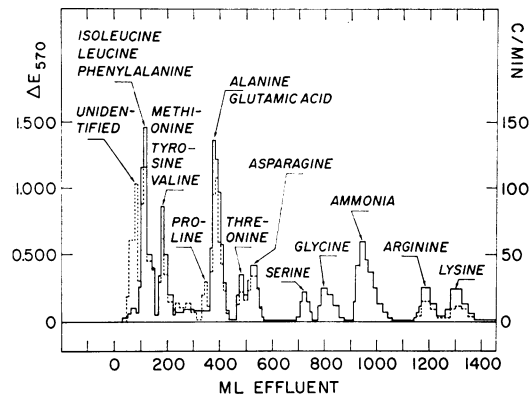
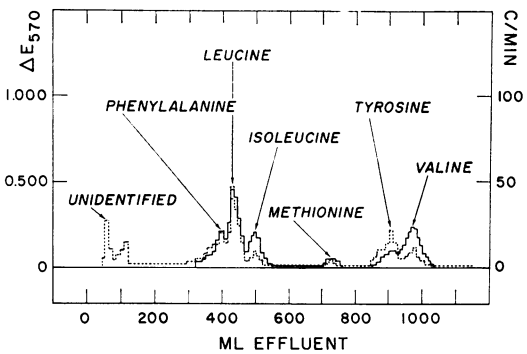


Figure 3. Chromatography of alcohol extracts on aluminum oxide. In this experiment the alcohol extracts from incubations with 70 g potassium acetate were combined. Composition of the reaction mixtures was as described in table 3. Solid line, absorption at 400 mμ; dotted line, radioactivity as counts per min per ml.





Figures 4 (top) and 5 (bottom). Starch column chromatography of protein hydrolyzate after incubation of dried cells with 2-C^{14} acetate. The columns, of 3-cm diameter, were prepared from 134 g anhydrous starch (Moore and Stein, 1949). The hydrolyzates were prepared by refluxing with 6 N HCl for 6 hr. The HCl was evaporated, and the residue dissolved in the solvent used for chromatography.

Figure 4. Hydrolyzate from 100 mg residue; solvent: *n*-butanol-*n*-propanol-0.1 N HCl (1:2:1).

Figure 5. Hydrolyzate from 75 mg residue; solvent: *n*-butanol-benzyl alcohol-water (1:1:0.288). Solid line: E_{570} after reaction of 0.2 ml eluate with ninhydrin (Moore and Stein, 1954). Broken line: radioactivity as counts per min per 0.2 ml eluate.

cent of the radioactivity adsorbed on the column, whereas fraction II contained 28 per cent. A brown band which remained at the top of the column could not be eluted. As shown in figure 3, the distribution of radioactivity in the eluate is associated with that of absorption at 400 mμ. The ratio of E_{400} to counts per min per ml is 1.5 to 1.8 × 10^{-4} in fraction I; this ratio in fraction II is not constant. Subsequent paper chromatography revealed that fraction I contained only the fluorescent component with the R_f value of 0.81, but fraction II contained both the R_f 0.42 and R_f 0.81 components.

After evaporating the solvent from fraction I, there remained an amber oil (0.8 g), which became solid on cooling to −10 C. This oil, soluble in alcohol, ether, chloroform, and hexane, and insoluble in water, acid, and alkali, could not be crystallized. The absorption spectrum of an alcohol solution of the oil showed increasing absorption from 550 to 230 mμ, without any significant peaks. The fluorescence spectrum shows peaks at 435 and 490 mμ with respective activation maxima at 352 and 395 mμ.

When fraction II was dried, a red oil remained (0.7 g), soluble in alcohol, chloroform, and alkali. Acidification of alkaline solutions resulted in separation as an oil. Attempts to prepare water-insoluble salts with heavy metals were not successful. The absorption spectrum of this fraction is similar to that of fraction I; the fluorescence spectrum is characterized by peaks at 450 and 470 mμ, with activation maxima at 352 and 395 mμ, respectively. In dilute solution this fraction has an intense odor of pine needles.

Analysis of the insoluble residue. Treatment of the water- and ethanol-insoluble residue described above with 6 N HCl resulted in solubilization of all radioactivity in the residue. This acid extract was then neutralized and passed over Dowex 50 (H^+ form); all radioactivity was absorbed, and it could be quantitatively eluted with 2 N ammonia. Paper chromatography revealed that the radioactivity was primarily in amino acids.

To ascertain the extent to which acetate was incorporated into individual amino acids, the protein hydrolyzate was subjected to starch column chromatography according to the method of Moore and Stein (1949). With a butanol-propanol-HCl solvent the hydrolyzate could be resolved into the single amino acids, threonine, asparagine, serine, glycine, arginine, and lysine, and two rapidly moving groups of amino acids (figure 4). The latter could be separated into individual amino acids by using a butanol-benzyl alcohol-water solvent system (figure 5). It is seen from these data that radioactive acetate was shown to be incorporated into almost every amino acid.

It was shown by Moore and Stein (1954) that the molar absorption coefficient for the ninhydrin-

TABLE 4

Distribution of radioactivity in the protein hydrolyzate after chromatography on starch columns

Fraction	Total Counts*	Ratio: Counts to E_{570}†
	%	
Unidentified compounds	17.5	—
Alanine and glutamic acid	21.5	70
Leucine	9.1	92
Phenylalanine	8.0	137
Asparagine	8.0	78
Tyrosine	6.5	170
Threonine	4.4	60
Arginine	4.2	62
Valine	3.8	28
Lysine	3.7	53
Proline	3.2	—
Isoleucine	2.3	48
Methionine	0.6	40
Glycine	0	0
Serine	0	0

* These values are expressed as percentage of the total radioactivity put on the column.

† Absorption at 570 mμ was measured after reaction of 0.2 ml eluate with ninhydrin. The ratio recorded here is counts per min per 0.2 ml eluate to E_{570} from 0.2 ml eluate.

amino acid complex is nearly identical for all these amino acids with the exception of proline. Therefore, the ratio of radioactivity to E_{570} would be a measure of the specific radioactivity of the individual amino acids, and would give some indication of which amino acids are preferentially synthesized from acetate in this system. Accordingly, the ninhydrin complexes of each of the separated amino acids were prepared and the ratio, counts per min to E_{570} measured. The results are presented in table 4, where it is seen that tyrosine and phenylalanine were preferentially synthesized. Of the branched-chain amino acids, only leucine showed a significantly greater than average incorporation.

Of the total radioactivity put on the starch column, 94 per cent is recovered in the various fractions. It is therefore concluded that most of the C^{14} incorporated into the insoluble residue represented conversion of acetate to amino acids. The presence of radioactivity in the fraction designated "unidentified compounds" in table 4 may be the result of decomposition of amino acids during the acid hydrolysis procedure (Duggan, 1957). Free ammonia which also was eluted from the column, was formed presumably by hydrolysis of amides and decomposition of amino acids.

ACKNOWLEDGMENTS

The author would like to thank Dr. Earl R. Stadtman and Dr. Thressa C. Stadtman for advice concerning the isolation of the organism and for many helpful suggestions during the course of this work.

SUMMARY

A new species of the genus *Pseudomonas* has been isolated from soil by the enrichment culture technique with citronellol as the sole carbon source. The morphological and nutritional characteristics of the organism are described, and the name *Pseudomonas citronellolis* n. sp. is proposed.

Dried cell preparations catalyze the oxidation of the isoprenoids farnesol and citronellol, as well as a number of related compounds which could be intermediates in isoprenoid degradation in this organism. Isotope studies on the metabolism of acetate in dried cells indicate that this substrate, a likely intermediate in isoprenoid degradation, is converted partially to ether-soluble fluorescent pigments as well as to protein amino acids. The purification of the pigments is described. Using chromatographic procedures it was shown that tyrosine, phenylalanine, and leucine were preferentially synthesized from acetate.

A method for the quantitative determination of citronellol and of citronellic acid is described.

REFERENCES

Bachhawat, B. K., Robinson, W. G., and Coon, M. J. 1956 Enzymatic carboxylation of β-hydroxyisovaleryl coenzyme A. J. Biol. Chem., **219**, 539–550.

Breed, R. S., Murray, E. G. D., and Smith, N. R. 1957 *Bergey's manual of determinative bacteriology*, 7th ed. Williams & Wilkins Co., Baltimore.

Duggan, E. L. 1957 Measurement of amino acids by column chromatography, In *Methods in enzymology*, vol. III, pp. 492–504. Edited by S. P. Colowick and N. O. Kaplan. Academic Press, Inc., New York.

Gould, R. G. 1958 Biosynthesis of cholesterol.

In *Cholesterol*, pp. 209–236. Edited by R. P. Cook. Academic Press, Inc., New York.

KNAPPE, J. AND LYNEN, F. 1958 Enzymatische Carboxylierung von β-methyl-crotonyl-Coenzyme A. Proc. Intern. Congr. Biochem., 4th Congr. (Wien), 49.

LIPMANN, F. AND TUTTLE, L. C. 1945 On the condensation of acetyl phosphate with formate or CO_2 in bacterial extracts. J. Biol. Chem., **158**, 505–519.

LYNEN, F., EGGERER, H., HENNING, U., AND KESSEL, I. 1958 Farnesyl-pyrophosphat and 3-Methyl-Δ^3-butiryl-1-pyrophosphat, die biologischen Vorstufen des Squalens (Zur Biosyntheses der Terpene, III). Angew. Chem., **70**, 738–742.

LYNEN, F., KNAPPE, J., LORCH, E., JUTTING, G., AND RINGELMANN, E. 1959 Die biochemische Funktion des Biotins. Angew. Chem., **71**, 481–486.

MOORE, S. AND STEIN, W. H. 1949 Chromatography of amino acids on starch columns. Solvent mixtures for the fractionation of protein hydrolyzates. J. Biol. Chem., **178**, 53–77.

MOORE, S. AND STEIN, W. H. 1954 A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J. Biol. Chem., **211**, 907–913.

SEMMLER, F. W. 1891 Ueber olefinische Bestandtheile aetherischer Oele. Ber. deut. chem. Ges., **24**, 201–211.

SMITH, L. I., PICHARD, W. V., AND SPILLANE, L. J. 1955 β,β-Dimethyl acrylic acid. In *Organic synthesis*, collective vol. III, pp. 302–304, Edited by E. C. Horning. John Wiley & Sons, Inc., New York.

Society of American Bacteriologists, Committee on Technic 1959 *Manual of microbiological methods*, p. 315. McGraw-Hill Book Co., Inc., New York.

STADTMAN, E. R. AND BARKER, H. A. 1949 Fatty acid synthesis by enzyme preparations of *Clostridium kluyveri*. V. A consideration of postulated 4-carbon intermediates in butyrate synthesis. J. Biol. Chem., **181**, 221–235.

STITT, E. R., CLOUGH, P. W., AND BRANHAM, S. E. 1948 *Practical bacteriology, hematology and parasitology*, 10th ed. The Blakiston Co., Philadelphia.