TABLE I

RADIOACTIVITIES OF HISTIDINE AND ASPARTIC ACID FRACTIONS FROM YEAST

	Histidine							Aspartic acid[a]			
	Content in yeast, %			Radioactivity,[a] counts/min. × 10^3			Ratio[b] Py/N_2	Radioactivity,[a] counts/min. × 10^3			Ratio[b] Py/N_2
	Py/O_2	Py/N_2	Ac/O_2	Py/O_2	Py/N_2	Ac/O_2	Py/O_2	Py/O_2	Py/N_2	Ac/O_2	Py/O_2
Whole molecule	1.2	1.3	1.1	8.7	6.1	1.8	0.70	25.4	20.6	15.0	0.81
Carboxyl (histidine)				0.48	0.66	0	1.38				
C_3 (aspartic acid)								1.80	2.15	0	1.19

[a] Expressed as counts/minute/mmole amino acid/mmole substrate utilized. [b] Obtained by dividing the specific activity of the whole molecule or particular atom derived from pyruvate anaerobically (under nitrogen) by the corresponding aerobically derived value.

In this Laboratory, radioactive histidine samples were isolated from bakers' yeast (*Saccharomyces cerevisiae*) grown on $CH_3C^{14}OOH$ aerobically and $CH_3C^{14}OCOOH$ both aerobically and anaerobically.[6] The isolations were carried out through ion-exchange resin column operations according to the method of Winters and Kunin.[7] The histidine samples were purified as a new derivative, the monoöxalate, which is readily recrystallized from aqueous ethanol and gives a definite decomposition point.

In the present work, preliminary degradation of the histidine samples has confirmed Ehrensvärd's observation using acetate; however a significant amount of C^{14} has been observed in the carboxyl group in histidine derived from pyruvate.

The specific radioactivities of histidine derived from each of the three experiments and the activities of the respective carboxyl groups are given in Table I.

From acetate, the histidine carboxyl is seen to be completely inactive, as shown previously.[3] The non-incorporation of acetate carboxyl into histidine carboxyl thus rules out the possibility of union of an imidazole ring with a C_3-unit (such as pyruvate) which might be produced through condensation of a C_2-compound with (radioactive) metabolic CO_2.

Among other possible intermediates in histidine biosynthesis, the C_4-acids deserve consideration. The pattern of labeling in oxalacetate, for example, may be presumed to be similar to the corresponding pattern in aspartic acid in this yeast.[8] In turn, the radioactivity of the methylene carbon (C_3) of aspartic acid furnished an index of histidine carboxyl (C_1) activity. Thus, from pyruvate, incorporation of C^{14} into both these atoms was similarly higher in the *anaerobic* sample (anaerobic/aerobic ratio = 1.38 in aspartate C_3, and 1.19 in histidine C_1) in spite of the fact that these amino acids as a whole showed greater specific radioactivity after *aerobic* growth. Finally, from acetate, both C_3 of aspartate and C_1 of histidine were unlabeled.

Experimental

Isolation of Histidine.—Histidine was isolated from hydrolyzed yeast protein previously freed from arginine, glutamic acid, aspartic acid, tyrosine and lysine.[9] The histidine, after a fivefold dilution with non-radioactive histidine, was adsorbed on an IRC-50 ion-exchange resin column (Rohm and Haas), buffered at *p*H 4.7 with sodium acetate, from which it was eluted with 4% hydrochloric acid solution. The eluate was repeatedly evaporated to dryness by distillation under reduced pressure to remove free hydrochloric acid and dissolved in water. An excess of silver nitrate was then added. The silver chloride was removed by centrifugation and the *p*H of the centrifugate adjusted to *p*H 7.4 to precipitate the silver salt of histidine. The washed silver salt was suspended in water and decomposed with hydrogen sulfide. Removal of the silver sulfide and evaporation of the filtrate under reduced pressure yielded crude histidine which was purified as the monoöxalate.

Purification of Histidine.—The histidine from the foregoing procedure (80–100 mg.) was dissolved in 2 to 4 ml. of water and mixed with a 100% excess of oxalic acid as a saturated solution. Alcohol was added to a concentration of approximately 80%. The white solid that formed was allowed to stand for 12 hours at 0°, then filtered and recrystallized by dissolving in water and reprecipitating with alcohol. The recrystallized material had a decomposition point beginning sharply at 223° (Fischer block, corrected). Over-all yields of 50 to 80% were experienced in this operation on a 100-mg. scale.

Anal. Calcd. for histidine monoöxalate: C, 39.2; H, 4.5; equiv. wt. of oxalate, 122.60; histidine, 63.4. Found: C, 39.9; H, 4.8; equiv. wt. of oxalate ($KMnO_4$), 122.5; histidine (microbiological assay), 67.

Radioactivity Measurements.—The specific radioactivities of histidine oxalate samples were determined by direct plating, using appropriate counting times for samples and backgrounds to maintain an over-all precision of 5%.[10] The carboxyl group specific radioactivities were determined by ninhydrin decarboxylation[11] after preliminary "cold" runs had established that satisfactory yields of barium carbonate could be obtained[4] and that oxalic acid gave no CO_2 with ninhydrin. The $BaC^{14}O_3$ thus obtained was plated and counted in the conventional manner with the usual correction for self-absorption applied.

(6) C. H. Wang, R. F. Labbe, B. E. Christensen and V. H. Cheldelin, *J. Biol. Chem.*, **197**, 645 (1952).

(7) J. C. Winters and R. Kunin, *Ind. Eng. Chem.*, **41**, 460 (1949).

(8) C. H. Wang, R. C. Thomas, V. H. Cheldelin and B. E. Christensen, *J. Biol. Chem.*, **197**, 663 (1952).

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(10) M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. F. Yankwich, "Isotopic Carbon," John Wiley and Sons, Inc., New York, N. Y., 1949, p. 288.

(11) *Ibid.*, pp. 260–261.

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Freezing Point and Vapor Pressure Data for Solutions of Carbon Dioxide with Some Halogen Substituted Methanes

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Vapor pressure–composition data were desired for liquid mixtures of carbon dioxide with bromochloromethane, dibromodifluoromethane and bromotrifluoromethane. The solutions were prepared by condensing successive measured volumes of carbon dioxide gas into the liquid organic compound which was in a bulb attached to a closed system and cooled by immersion in a *n*-propyl

alcohol-bath. The temperature was adjusted to the desired temperature by means of liquid nitrogen introduced into a coil of copper tubing immersed in the bath liquid. The solution in the bulb was stirred by a helix of wire whose up and down motion was magnetically controlled. The pressure in the system was measured with a mercury manometer. The volume of the system was known in order to apply a correction for uncondensed gases. The temperature when crystals first appeared in the solution upon cooling was measured with a single junction copper–constantan thermocouple and a Rubicon, type B, precision potentiometer.

TABLE I

FREEZING POINTS OF BROMOCHLOROMETHANE–CARBON DIOXIDE SYSTEM

Mole % CO_2	F.p., °C.	Eutectic	Press., cm.
0	−87.9		0.2
4.6	−89.9		14.8
6.0	−90.5		19.8
8.4	−91.3		21.9
10.6	−92.1	−92.1	23.6
12.1	−90.4	−92.1	31.1
15.0	−85.5	−92.1	48.0
21.6	−80.0		79.3
29.2	−73.8		122.6
38.8	−69.7	−92.1	160.7
60[a]	−64.2		242
80[a]	−60.3		316
100[b]	−56.6		388.5

[a] Estimated from smooth curve. [b] "International Critical Tables," Vol. III, McGraw-Hill Book Co., Inc., New York, N. Y., 1928, p. 235.

TABLE II

FREEZING POINTS OF DIBROMODIFLUOROMETHANE–CARBON DIOXIDE SYSTEM

Mole % CO_2	F.p., °C.	Eutectic	Press., cm.
0	−141.6		0.5
0.7	−141.4	−142.5	.4
2.3	−127.7	−142.7	.4
5.0	−114.8	−142.7	2.8
9.7	−104.0		11.4
16.2	− 93.2		27.7
20.1	− 88.2		38.1
28.2	− 80.7		78.9
41.7	− 74.6	−142.6	111.00
47.2	− 71.7		145.0
60[a]	− 67.3		193
80[a]	− 61.4		289
100[b]	− 56.6		388.3

[a] See footnote *a* in Table I. [b] See footnote *b* in Table I.

TABLE III

FREEZING POINTS OF BROMOTRIFLUOROMETHANE–CARBON DIOXIDE SYSTEM

Mole % CO_2	F.p., °C.	Press., cm.	°C. for 76 cm.
0	−168	5	−64.7
0.8	−154.2	4.5	
1.1	−150.9		
2.0	−137.0		
4.9	−121.8	8.4	
10.0	−107.6	21.0	−74.7
22.2	− 92.7	49.2	−81.7
33.8	− 83.7	79.0	−84.1
46.7	− 76.9	121.7	
60[a]	− 71.0	187	−84.0
80[a]	− 62.9	285	−81.7
100[b]	− 56.6	388.5	−78.5

[a] See footnote *a* in Table I. [b] See footnote *b* in Table I.

Materials.—The bromochloromethane was purified by distillation in a multiple plate fractionating column; b.p. 68.0° at 76 cm.; f.p. −87.9°. The dibromodifluoromethane after several distillations had a freezing point of −141.6°. The bromotrifluoromethane as procured contained about 10% carbon dioxide as impurity. After purification a solidification temperature of −168 ± 2° was obtained.

Results.—The data are presented in Tables I–III. For the system CH_2BrCl–CO_2, the eutectic temperature is −92.1° at 10.6 mole per cent. CO_2; for the system CBr_2F_2–CO_2, −142.6° at 0.5 mole per cent. CO_2; and for the system $CBrF_3$–CO_2, at −170° at a concentration less than 0.5 mole per cent. CO_2.

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Terpenoids. I. The Triterpenes of the Cactus *Lemaireocereus Thurberi*

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In connection with a study now under way in this Laboratory[2] on the alkaloids of certain cacti, it appeared of interest also to investigate the non-basic constituents of certain members of the *Cactaceae* family. The present note is concerned with such an examination of the cactus *Lemaireocereus Thurberi*, the only species of the genus *Lemaireocereus*, the natural habitat of which extends as far north as Arizona.[3] This cactus, reaching up to 21 feet in height, is particularly abundant in northern Mexico in the states of Sonora, Nayarit, Sinaloa and Baja California where it is known as "pitahaya dulce"[4] and it is often used by the natives for natural fences. The specimens employed in the present study were obtained through the cooperation of Dr. R. R. Humphrey, University of Arizona, who collected them near Hermosillo, Sonora.

A chemical study of the alcoholic extract of the dried and pulverized plant indicated the total absence of alkaloids, in marked contrast to the abundance of alkaloids in some related genera.[2] Similarly, the neutral portion yielded only negligible amounts of crystalline material, but there was an appreciable, water-soluble, glycosidic fraction. Acid hydrolysis of this material yielded a neutral and an acidic component. The former appears to be an unknown triterpene ($C_{30}H_{46}O_3$), the infrared spectrum (Fig. 1) of which showed bands at 2.80 and 5.65 μ characteristic of a free hydroxyl group and a five-membered lactone ring. The substance formed a monoacetate with infrared carbonyl

(1) Syntex Post-doctorate Fellow, 1952–1953.

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(3) N. L. Britton and J. N. Rose, "The Cactaceae," Vol. II, p. 98, Carnegie Institution of Washington, Washington, D. C., 1920.

(4) H. Bravo, "Las Cactaceas de Mexico," Mexico, D. F., 1937, p. 269.