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2-Aminoadamantane-2-carboxylic Acid, a Rigid, Achiral, Tricyclic α-Amino Acid with Transport Inhibitory Properties[1,†]

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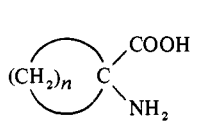
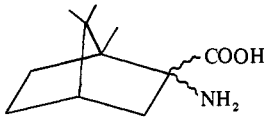
2-Aminoadamantane-2-carboxylic acid, a geminally functionalized, achiral, tricycloaliphatic aminocarboxylic acid, was synthesized from adamantan-2-one *via* the Bucherer–Lieb hydantoin procedure. The adamantylamino acid inhibited the transport of L-leucine and L-methionine into Ehrlich ascites cells *in vitro* and, on a molar basis, was a better inhibitor in this system than cycloleucine. The theoretical structural requirements for transport by synthetic aliphatic amino acids proposed by Tager and Christensen appear to apply also to transport inhibition.

1-Aminocyclopropanecarboxylic acid (**1a**), a geminally functionalized cycloaliphatic aminocarboxylic acid, occurs naturally in the cowberry[2] and in perry pears and cider apples.[3] Ring homologs of **1a**, *viz.*, **1b–f**, as well as the bicyclic **2**, have been synthesized[4,5] and tested in a number of tumor systems,[6] but only 1-aminocyclopentanecarboxylic acid (cycloleucine, **1c**) showed sufficient antitumor activity to reach clinical trials.[7] A nitro-substituted **1c**, *viz.*, 1-amino-2-nitrocyclopentanecarboxylic acid, which showed unusual plant growth regulating properties, has been isolated from the fermentation culture filtrates of *Aspergillus wentii*.[8]



1a, n = 2
b, n = 3
c, n = 4
d, n = 5
e, n = 6
f, n = 7



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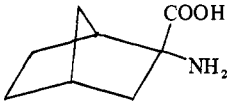
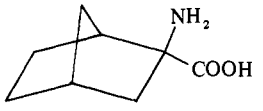


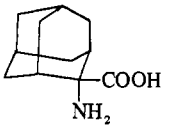


3

2-Amino-2-bornanecarboxylic acid (**2**)[5] represents a system where the geminal amino and carboxyl groups are anchored to a rigid, naturally occurring bicyclic terpene. A similar rigid system obtains in the isomeric 2-aminobicyclo-[2.2.1]heptane-2-carboxylic acids (**3**) synthesized by Christensen, *et al.*[9]

Cycloaliphatic α-amino acids such as represented by cycloleucine (**1c**) and the bicyclic **3** are neither metabolized *in vivo* nor incorporated into tissue proteins[10,11] but are actively transported by the transport system serving for the natural amino acids with apolar side chains.[9] They competitively inhibit the uptake of valine, leucine, and methionine into Ehrlich ascites tumor cells, this property constituting a possible mechanism of action for the antineoplastic activity of **1c**.[12]

The theoretical structural requirements for transport by these synthetic aliphatic amino acid analogs have been summarized recently by Tager and Christensen[13] to be (a) side chain bulk in all dimensions for minimal interaction with other transport systems, (b) maximally apolar side chains to promote high affinity for the transport system, (c) a tertiary α-carbon atom to impart resistance to catabolism, and (d) sufficient water solubility. Criteria a, b, and c appear to be fulfilled by the tricyclic 2-aminoadamantane-2-carboxylic acid (**4**),‡ the title compound, a rigid, achiral α-amino acid, and the expected zwitterionic character of **4** would appear to fulfill criterion d.



4

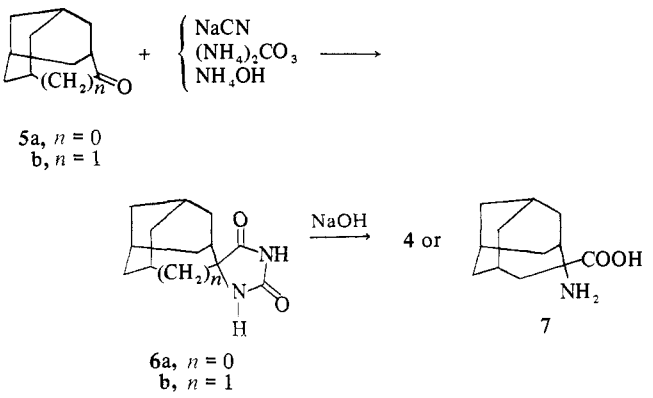
Chemistry. α-Amino acids having an adamantane skeleton as an integral part of the molecule have not yet been reported.[1] The Strecker synthesis to **4** was initially attempted starting from adamantan-2-one (**5a**) *via* its cyanohydrin and the corresponding aminonitrile, but hydrolysis of the latter to **4** could not be effected without extensive by-product formation, and this route was abandoned in favor of the Bucherer–Lieb synthesis as outlined in Scheme I. Although elevated temperatures and pressures were necessary to effect condensation to the spirohydantoin **6a** and similar high temperatures were required to hydrolyze **6a** to **4** (see Experimental Section), the overall yield of **4** from adamantan-2-one (**5a**) was 81%.

The electron-impact (EI) mass spectrum of **4** displayed a prominent $(M - CO_2H)^+$ fragmentation peak at m/e 150, with a molecular ion at m/e 195 of only feeble intensity as

†Presented in part at the 161st National Meeting of the American Chemical Society, Los Angeles, Calif., March 31, 1971.

‡Suggested trivial name: adamantan*ine*.

Scheme I



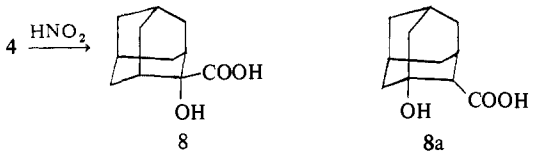
5a, $n = 0$
b, $n = 1$

6a, $n = 0$
b, $n = 1$

7

are typical of the EI spectra of most free α-amino acids and especially so for cycloleucine (**1c**).[14] Its molecular weight and structure were further verified by the isobutane chemical ionization (CI) mass spectrum which showed the presence of a quasimolecular ion $(MH)^+$ at *m/e* 196 and the fragments $(MH - NH_3)^+$ at *m/e* 179 and $(MH - CO_2H_2)^+$ at *m/e* 150. These are expected fragments for an α-amino acid.[15] The EI and CI mass spectra of the *N*-trifluoroacetyl deriviative of **4** were likewise consistent with the structural assignment for **4**.

Nitrous acid deamination of **4** gave a remarkably good yield (94%) of the corresponding 2-hydroxyadamantane-2-carboxylic acid (**8**) in view of the known propensity for carbonium ion formation in the adamantane series[16] and the possibility for rearrangement or ring fission reactions, *e.g.*, as in the Schmidt reaction of **5a** or the Beckmann rearrangement of its oxime.[17-20] Dehydration of **8** is, of course, not possible[16] without prior ring fission. That the



8 8a

product was **8** and not the rearranged 1-hydroxy isomer **8a** was adduced by the identity of its melting point as well as its ir and nmr spectra with the product described by Liggero, *et al.*[21] Moreover, its EI mass spectrum showed a prominent $(M - CO_2H)^+$ peak at *m/e* 151 [which can be considered the oxygen equivalent of the $(M - CO_2H)^+$ peak for **4**] consistent with structure **8**.

The insolubility of **4** in H_2O at room temperature over the pH range 2.8-8.8 is notable in view of its infrared spectrum which showed characteristic α-amino acid zwitterionic character. Although **4** could be recrystallized from H_2O, its solubility was so limited that even hot 1.5 m*M* solutions deposited crystals on cooling. **4** was also essentially insoluble in hot organic solvents such as EtOH, $CHCl_3$, EtOAc, or DMF.

In an effort to circumvent this lack of solubility of **4**—a property very likely related to its extreme symmetry and which presented problems in biological evaluations (*vide infra*)—the homologous amino acid **7** was prepared from homoadamantan-4-one (**5b**) *via* the spirohydantoin **6b** as in Scheme I. No longer achiral, the presence of the additional methylene group in **7** transforms one of the tricyclic ring system into a seven-membered ring. However, no great advantage was gained and any increase in solubility of **7** over **4** was marginal at best.

Biological Activity. For toxicity studies it was necessary to administer the amino acid **4** as a finely pulverized suspension in H_2O using Tween-20 as dispersing agent. Use of the hydrochloride of **4**, which was considerably more water soluble, was precluded by the observation that even a 1.5 m*M* aqueous solution was acidic (pH 2.6) and buffering to physiological pH's caused the precipitation of **4**. Rats (Sprague-Dawley) tolerated single oral doses of 1000 mg/kg ($n = 4$) and single ip doses of 500 mg/kg ($n = 4$) without observable toxicity. The 96-hr LD_{50}[22] for a single ip dose to HA/ICR mice (approximately 400-700 mg/kg) could not be determined accurately even though repeated many times as the values appeared to depend on particle size. Indeed, aggregates of amino acid particles (confirmed by ir) which were enveloped in membranous material were found at necropsy in the peritoneal cavities of mice receiving the higher doses. The observed delayed toxic deaths of 3-4 days are very likely due to this repository action.

The inhibition by **4** of the transport of L-leucine-*1*-^{14}C and [methyl-^{14}C]-L-methionine into Ehrlich ascites tumor cell system was evaluated *in vitro*. These data are recorded in Figures 1-4. The solubility problems alluded to above prevented the use of concentrations of **4** greater than 0.5 m*M* and frustrated attempts to achieve greater inhibition by utilizing concentration levels attainable with cycloleucine (**1c**), a known inhibitor in this system.[12,23,24] As with cyclo-

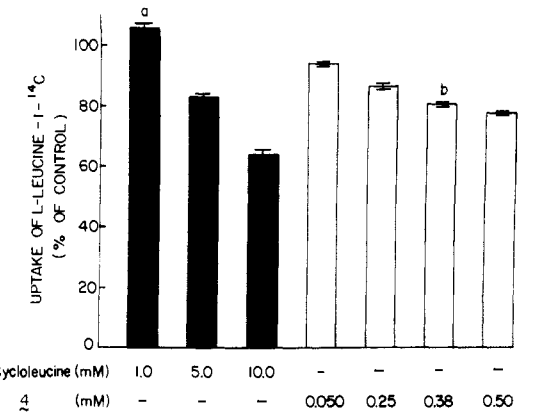


Figure 1. Inhibition of leucine-*1*-^{14}C uptake into Ehrlich ascites cells by 2-aminoadamantane-2-carboxylic acid (**4**) compared against cycloleucine (**1c**). The conditions are described in the Experimental Section, and the results of triplicate determinations (±S.D.) are expressed relative to controls. (a) The stimulation of uptake seen here at this concentration of cycloleucine has been described (ref 23). (b) Duplicate determinations only.

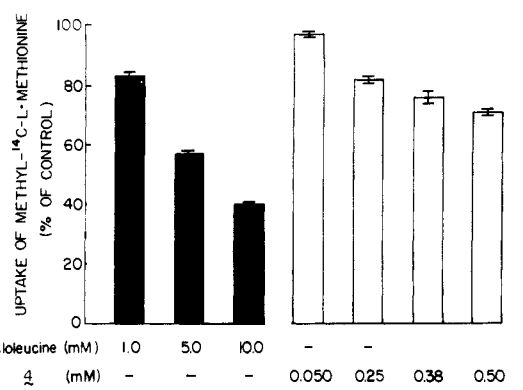


Figure 2. Inhibition of [methyl-^{14}C]methionine uptake into Ehrlich ascites cells by 2-aminoadamantane-2-carboxylic acid (**4**) compared against cycloleucine (**1c**). Details as in Figure 1.

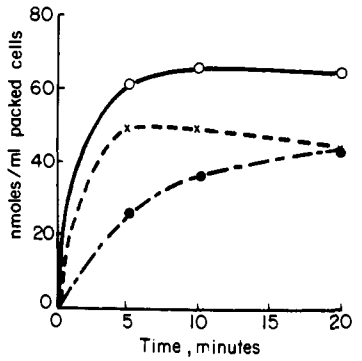


Figure 3. Time course of [methyl-^{14}C]methionine uptake into Ehrlich ascites cells in the presence of (•) 10 m*M* cycloleucine, (×) 0.50 m*M* 4, and (○) without inhibitor. Alcohol-soluble radioactivity.

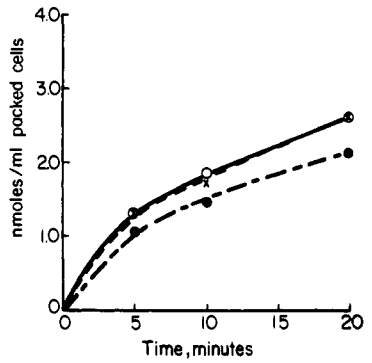


Figure 4. Details as in Figure 3. Alcohol-insoluble radioactivity.

leucine, the inhibition of uptake of leucine-*l*-^{14}*C* and [methyl-^{14}C]methionine into Ehrlich ascites cells by **4** was concentration dependent (Figures 1 and 2). Comparison of the apparent K_i values for methionine uptake for **4** (0.76 m*M*, calculated from the 5-min values using K_m of 1.76 m*M*[23]) with that of cycloleucine (4.4 m*M*) showed that the transport inhibitory activity of **4** was approximately 5.8 times greater than that of cycloleucine in this system. Comparison of the $[I/S]_{0.5}$ values gave a ratio of 5.5.

Whereas the inhibition of methionine uptake by cycloleucine was rapid in onset, being maximal below 5 min, the inhibition by **4** reached maximal levels only after 20 min of incubation at which time the level of inhibition reached that observed for cycloleucine (Figure 3). The 20-fold higher concentration of cycloleucine *vis-à-vis* **4** should be noted here. The incorporation of radioactivity into the alcohol-insoluble fraction of ascites cells was not inhibited by **4**, and it appears that protein synthesis was unaffected (Figure 4). The slight inhibition seen here with cycloleucine can be ascribed to the early inhibition of transport of methionine into the cells by this inhibitor.[12,23,24]

The biological properties of the homologous tricyclic α-amino acid **7** were not studied *in extenso*, but **7** at 1.0 m*M* did not significantly inhibit the uptake of L-leucine-*l*-^{14}*C* into Ehrlich ascites cells and, in fact, stimulated the uptake of [methyl-^{14}C]-L-methionine by 12–13% under conditions where **4** was inhibitory (80% of control).

Although the data of Figures 1 and 2 might predict an antitumor activity for **4** *in vivo*, this was not observed. Thus, **4** did not inhibit the growth of L1210 lymphoid leukemia in host BDF_1 mice when given intraperitoneally (suspension in alcohol) every fourth day for a total of three doses up to 400 mg/kg per dose. Likewise, **4** did not inhibit the growth of P388 lymphocytic leukemia in the above treatment protocol (200 mg/kg × 3 in alcohol or 100 mg/kg × 3 in hydroxypropylcellulose).

Discussion

The validity of the structural parameters required for transport by synthetic aliphatic α-amino acids as proposed by Tager and Christensen[13] appears to be verified by the high transport inhibitory activity observed here for 2-aminoadamantane-2-carboxylic acid (**4**) which, by *a priori* considerations, fulfilled essentially all criteria. The insolubility of **4** in H_2O—for which we have no good explanation—was quite unexpected.§ Like cycloleucine itself, the absence of a chiral center assures its ready synthetic availability and should faciliate biochemical interpretations without ambiguity. As might be predicted, the isomeric norbornylamino acids **3** possess different transport properties.[9]

A more detailed investigation of the transport properties of **4** (as opposed to transport inhibitory properties), *i.e.*, whether it is itself concentrated by Ehrlich ascites cells *in vitro*, and of its metabolism *in vivo* requires radioisotopically labeled **4**. This should be readily accessible *via* Scheme I using NaCN-^{14}*C* or, for ring-labeled **4**, by starting with 2-^{14}C-labeled **5a**.[21] However, the absence of favorable antitumor activity of **4** *in vivo* discouraged further studies along these lines.

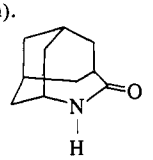
Experimental Section

Melting points (corrected) were determined in a Mel-Temp apparatus in sealed capillary tubes with the head space above the sample filled with glass rod to minimize sublimation. Spectrophotometers used were: ir, Beckman IR-10; nmr, Varian A-60; mass spectrum (EI, ionization energy, 70 eV, ion source temperatures as indicated) Hitachi Perkin-Elmer, RMU-6; chemical ionization mass spectra (CI) were provided by Dr. Roger Foltz, Battelle Columbus Laboratories, Columbus, Ohio, using an AEI-MS-902 mass spectrometer equipped with an SRIC Model CIS-2 combined CI-EI ion source. Radioactivity was measured in a Packard Model 3375 liquid scintillation spectrometer. Microanalyses were carried out by Galbraith Laboratories, Knoxville, Tenn. Mice bearing Ehrlich ascites tumors were obtained from Microbiological Associates, Bethesda, Md., and the tumor line was maintained by inoculating mice every fourth or fifth day.

Spiro(adamantane-2,4'-imidazolidine)-2',5'-dione (6a). A mixture of adamantan-2-one (**5a**) (15.02 g, 0.10 mol), NaCN (11.0 g, 0.22 mol), $(NH_4)_2CO_3 \cdot H_2O$ (17.1 g, 0.15 mol), EtOH (200 ml), and concentrated NH_4OH (200 ml) was charged into a 1-l. stainless steel pressure reaction vessel. After heating 3 hr with stirring at 120° and 12 kg/cm² (170 psi) of pressure, the reaction mixture was cooled and diluted with H_2O (500 ml) and the solids were collected. The solids were washed with H_2O, acetone, and Et_2O and air-dried to yield dense white crystals: mp 296–299°; 21.80 g (99% yield). The analytical sample was recrystallized from tetrahydrofuran, mp 296–299°. *Anal.* ($C_{12}H_{16}N_2O_3$) C, H, N.

2-Aminoadamantane-2-carboxylic Acid (4). A suspension of the crude hydantoin **6a** prepared above in 1.25 *N* NaOH solution (400 ml) in a 1-l. stainless steel pressure vessel was heated 2 hr with stirring at 195° and 17.5 kg/cm² (250 psi) of pressure. The cooled reaction mixture was diluted with H_2O (800 ml) and the solution acidified with concentrated HCl. Addition of acid was continued until the precipitate which formed initially had redissolved (pH 1.5). The solution was decolorized with charcoal and slowly neutralized with 2 *N* NaOH, whereupon fine white plates crystallized.

§ *E.g.*, the lactam **i** (ref 25) derived from the Beckmann rearrangement of the oxime of **5a** has a solubility in H_2O at 25° of >0.3 *M* (our published observation).



i

The product was collected, washed with H_2O, acetone, and Et_2O, and dried *in vacuo* over P_2O_5 overnight to give 15.95 g (81% overall yield from **5a**) of analytically pure **4**, mp 308–310° dec. *Anal.* ($C_{11}H_{17}NO_2$) C, H, N. Recrystallization from H_2O raised the melting point to 312–314° dec: ir (KBr) 1560 cm^{-1} (COO^-); mass spectrum (EI, 350°) *m/e* (rel intensity) 195 (<0.5, $M\cdot^+$), 150 (100, $\%\Sigma_{39}$ 37.4, $[M - CO_2H]^+$); mass spectrum (CI, isobutane, 200°) *m/e* (rel intensity) 196 (100, MH^+), 179 (18, $[MH - NH_3]^+$), 150 (61, $[MH - CO_2H_2]^+$). *Anal.* ($C_{11}H_{17}NO_2$) C, H, N. The hydrochloride of **4** (by concentrating a solution of **4** in dilute HCl) had mp dec above 280°; ir (KBr) 1732 cm^{-1} (COOH). *Anal.* ($C_{11}H_{18}NO_2Cl$) C, H, N. The *N*-**trifluoroacetyl derivative** of **4** was prepared by heating **4** with $(CF_3CO)_2O$ in $CHCl_3$ at 50°: recrystallized from CH_2Cl_2; mp 208–210° with dec; ir (KBr) 3200 (NH), 1725 (COOH), 1705 (amide I), 1500 cm^{-1} (amide II); mass spectrum (EI, 150°) *m/e* (rel intensity) 291 (0.4, $M\cdot^+$), 246 (100, $[M - CO_2H]^+$), 178 (21.7, $[M - CF_3CONH_2]\cdot^+$); mass spectrum (CI, isobutane) *m/e* (rel intensity) 292 (15, MH^+), 274 (5, $[MH - H_2O]^+$), 246 (100, $[MH - CO_2H_2]^+$). *Anal.* ($C_{13}H_{16}NO_3F_3$) C, H, N.

Spiro(imidazolidine-4,4′-tricyclo[4.3.1.1^{3,8}]undecane)-2,4-dione (6b). A mixture of homoadamantan-4-one (**5b**)[26] (16.40 g, 0.10 mol), NaCN (10 g, 0.20 mol) and $(NH_4)_2CO_3\cdot H_2O$ (25 g, 0.22 mol), EtOH (100 ml), and concentrated NH_4OH (75 ml) was heated at 170° and 14.1 kg/cm^2 (200 psi) of pressure for 3 hr. The cooled reaction mixture was diluted with H_2O (1500 ml), and the solids were collected, washed with H_2O, and used directly in the next reaction. For analysis, a small sample of the product was recrystallized from MeOH to give colorless plates, mp 306–309°. *Anal.* ($C_{13}H_{18}N_2O_3$) C, H, N.

4-Aminohomoadamantane-4-carboxylic Acid (7). The bulk of the crude hydantoin 6b above was hydrolyzed in 2 *N* NaOH (200 ml) by heating for 2 hr at 130° and 12 kg/cm^2 (170 psi). After cooling, the reaction mixture was worked up as for **4** above. The overall yield from homoadamantan-4-one was 5.20 g (25%), mp 293–295° dec. The analytical sample was recrystallized twice from MeOH–H_2O, mp 297–298° dec. *Anal.* ($C_{12}H_{19}NO_2$) C, H, N.

2-Hydroxyadamantane-2-carboxylic Acid (8). To a solution of 1.16 g (5.9 mmol) of **4** in 100 ml of 3 *N* HCl was added a solution of 2.0 g (0.03 mol) of $NaNO_2$ in 10 ml of H_2O. After stirring at room temperature for 2 hr, the solids which precipitated were collected, washed with H_2O, and recrystallized from $CHCl_3$–ether to give 1.10 g (94% yield) of colorless plates, mp 211–212.5° (reported[21] mp 210–211°). The ir (KBr) and nmr (CD_3OD, TMS) spectra were as reported;[21] mass spectrum (EI, 240°) *m/e* (rel intensity) 196 ($M\cdot^+$, <0.5), 151 (100, $\%\Sigma_{39}$ 32.9). *Anal.* ($C_{11}H_{16}O_3$) C, H.

Transport Inhibition Studies. Male white mice [$(HA/ICR)_f$, 3–4 weeks old] were inoculated ip with 0.2 ml of Ehrlich ascites fluid diluted 1:5 with saline. After 5 days the ascites cells were isolated according to Johnstone and Scholefield.[27] For transport studies, the procedure of Ahmed and Scholefield[24] was modified as follows. Each incubation mixture (25 ml, erlenmeyer flask) in a total volume of 3.0 ml of calcium-free Krebs–Ringer phosphate (KRP) buffer (pH 7.4) contained (final concentrations): 1.0 m*M* L-leucine-*1*-^{14}C or 1.0 m*M* [methyl-^{14}C]-L-methionine and cycloleucine (1c) or 2-aminoadamantane-2-carboxylic acid (**4**) as inhibitors at concentrations indicated in Figures 1–4. Controls contained no inhibitors. Uptake was initiated by the addition of 1.0 ml of 1:12 diluted (KRP) packed Ehrlich ascites cells and the stoppered flask incubated at 37° for 5.0 min in a shaking water bath. The uptake was quenched by decanting the contents of the flask into a centrifuge tube containing 5.0 ml of cold KRP buffer using an additional 2.0 ml for wash. The cells were immediately sedimented at top speed in a table-top centrifuge for 1 min and the supernatant fluid was decanted. The side of the inverted tube was wiped successively with KRP-moistened gauze followed with a dry gauze and the intracellular radioactivity extracted by suspending the cells (vortex stirrer) in 3.0 ml of 95% EtOH and allowing to stand for 1 hr at room temperature. After centrifugation, the soluble radioactivity in the supernatant was counted in a diotol[28] system. The alcohol-insoluble residue was washed and dried,[27] dissolved in a commercial protein solubilizer (Nuclear Chicago Corp.), and counted in a toluene medium containing 0.6% PPO and 0.025% POPOP after addition of 25 μl of glacial HOAc for color dispersal. The total intracellular radioactivity was expressed as nanomoles of leucine-^{14}C (or methionine-^{14}C) taken up per milliliter of packed cells in 5 min.

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