occurred by mere storage of synthetic Δ^9THC, dissolved in cyclohexane, for several months in the frozen state. The absence of the Δ^8THC band in our chromatograms of smoke condensates supports the finding of Claussen and Korte (*10*) and of Lerner and Zeffert (*11*) that isomerization of Δ^9THC into Δ^8THC occurs only to a very small extent under smoking conditions.

Extracts of thyme, oregano, sage, rosemary, clove, caraway seeds, alfalfa, tobacco, menthol, green tea, and rhubarb leaves were chromatographed because these substances reportedly have been used to dilute or substitute for marihuana (*4*). Thyme, clove, oregano, and thymol each formed a single band which migrated about 20 mm. These bands did not mask those of cannabinoids, alter migration, or interfere with identification when mixed with the synthetic standards. The other extracts in this group did not produce detectable bands.

It is expected that, in addition to its value for qualitative work, the technique described will be useful for purification of components for structural analysis by other methods.

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References and Notes

1. R. Mechoulam, *Science* **168**, 1159 (1970).
2. E. Ribi, C. Filz, K. Ribi, G. Goode, W. Brown, M. Niwa, R. F. Smith, *J. Bacteriol.* **102**, 250 (1970).
3. E. Ribi, C. J. Filz, G. Goode, S. M. Strain, K. Yamamoto, S. C. Harris, J. H. Simmons, *J. Chromatogr. Sci.* **8**, 577 (1970). An apparatus for centrifugal chromatography is available from Ivan Sorvall, Inc., Newton, Conn. 06470.
4. M. J. de Faubert Maunder, *J. Pharm. Pharmacol.* **21**, 334 (1969); H. D. Beckstead and W. N. French, *Microgram* **2**, 4 (1969); U. Claussen and F. Korte, *Liebigs Ann. Chem.* **713**, 166 (1968); B. Caddy and F. Fish, *J. Chromatogr.* **31**, 584 (1967); J. Betts and P. J. Holloway, *J. Pharm. Pharmacol.* **19** suppl., 97S (1967); J. Kolsek, H. Matičič, R. Repič, *Arch. Pharmacol.* **295**, 15 (1962); R. S. DeRopp, *J. Amer. Pharm. Ass. Sci. Ed.* **49**, 756 (1960).
5. H. Aramaki, N. Tomiyasu, H. Yoshimura, H. Tsukamoto, *Chem. Pharm. Bull. Tokyo* **16**, 822 (1968).
6. F. Korte and H. Sieper, *J. Chromatogr.* **13**, 90 (1964). The alkalinity of residual diethylamine in the chromatograms was sufficient to provide reproducible color reactions; therefore it was not necessary to add sodium hydroxide to the spray reagent. A Desaga 100-ml atomizer (No. 25 09 47, Brinkmann Instruments, Inc., Westbury, Long Island, N.Y. 11590) was used for spraying the chromatograms because it provided better results than several others tested.
7. ———, *J. Chromatogr.* **14**, 178 (1964).
8. Apart from our comparison of migration distances and specific color reactions, the identification of natural Δ^9THC and CBN was further supported by the correlation of ultraviolet spectra between natural cannabinoids eluted from chromatograms and artificial standards.
9. J. W. M. Davis, C. G. Farmilo, M. Osadchuk, *Anal. Chem.* **35**, 751 (1963); U. Claussen, W. Borger, F. Korte, *Liebigs Ann. Chem.* **693**, 158 (1966).
10. U. Claussen and F. Korte, *Tetrahedron Lett.* **22**, 2067 (1967); *Liebigs Ann. Chem.* **713**, 162 (1968).
11. M. Lerner and J. T. Zeffert, *Bull. Narcotics* **20**, 53 (1968).
12. We thank Dr. J. A. Scigliano, Center for Studies of Narcotics and Drug Abuse, National Institute of Mental Health, Chevy Chase, Md., for the synthetic cannabinoid standards; the Montana Tuberculosis Association for support of S.M.S.; and D. E. Dye, Sheriff of Ravalli County, Montana, for confiscated marihuana and hashish.

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Ascorbate Sulfate: A Urinary Metabolite of Ascorbic Acid in Man

Abstract. *Ascorbate-3-sulfate is a significant metabolite of ascorbic acid excreted in human urine. The characteristics of this compound were determined in experiments in which labeling with carbon-14 and tritium was used coupled with cochromatography with synthetic ascorbate-3-sulfate (both labeled and not labeled with sulfur-35) in a variety of solvent and absorbent systems.*

Little information is available on urinary metabolites of ascorbic acid in man. Oxalate has been identified (*1*). The only other known excretion product is ascorbate itself. In this report, we describe the identification of ascorbate-3-sulfate in urine of human subjects with scurvy (*2*).

Male volunteers fed a diet deficient in ascorbic acid were given [1-^{14}C, 4-^{3}H]ascorbate (*2*). Urine from these subjects was fractionated into a lead acetate precipitate at *p*H 4 and *p*H 8, and a soluble (S) fraction. The presence of both ^{14}C and ^{3}H in each fraction indicated a variety of labeled metabolites. The percentage of ^{14}C in the S fraction was markedly lower at the 9th week for one subject with signs of severe scurvy at that time. A qualitative correlation between the degree of scurvy and the percentage of ^{14}C in the S fraction was observed in all five subjects in this study (*2*).

The S fractions were examined by electrophoresis in a 0.05*M* ammonium acetate buffer (*p*H 5.8). The major component in this fraction proved to have an electrophoretic mobility slightly less than picrate ($R_{picrate} = 0.78$) and a ratio of ^{14}C to ^{3}H the same as that of the administered labeled ascorbate. These properties show that this metabolite was a strong acid in which the carbon chain was almost certainly intact. Furthermore, the compound must have had an unusual stability since the urine samples, stored in frozen acidified solution, were almost 2 years old at the time of analysis.

Experiments were undertaken to show whether the metabolite was ascorbate sulfate, such as has been described by Mead and Finamore for brine shrimp cysts (*3*) and synthesized by Chu and Slaunwhite (*4*). Ascorbate-3-sulfate was synthesized by a variation of their method and purified by barium salt precipitation and chromatography on a diethylaminoethyl (DEAE) cellulose–sulfate ion exchange column eluted with a linear gradient of dilute sulfuric acid. This procedure permits separation of the several ascorbate monosulfates. The reference

Table 1. Cochromatography of synthetic and urinary ascorbate-3-sulfate. The composition of solvent systems was as follows; all proportions are by volume. Pabst III: 0.1*M* potassium phosphate buffer (*p*H 6.8), ammonium sulfate, and 1-propanol (10 : 6 : 2), ascending paper; BAW: 1-butanol, acetic acid, and water (5 : 2 : 3 or 6 : 4 : 3), descending paper; AFI: 0.04*M* ammonium formate buffer (*p*H 4.3) and 2-propanol (6 : 4), ascending thin-layer chromatography (TLC); EAW: ethylacetate, acetic acid, and water (6 : 3 : 2 or 5 : 2 : 3), ascending TLC; CMW: chloroform, methanol, and water (65 : 25 : 4), ascending TLC; ABW: acetonitrile, butyronitrile, and water (66 : 33 : 2), ascending TLC; EPW: ethylacetate, pyridine, and water (8 : 2 : 1), ascending TLC; BB: methylethylketone, acetic acid, and saturated boric acid (9 : 1 : 1), ascending TLC.

Absorbent	Solvent	R_F	
		Synthetic	Urinary
Whatman No. 1 paper	Pabst III	0.89	0.90
Whatman No. 1 paper	BAW	.52	.54
Bakerflex cellulose	AFI	.65	.64
Bakerflex cellulose	EAW	.39	.39
Silica gel H	ABW	.0	.0
Silica gel H	EPW	.0	.0
Silica gel H	BB	.0	.0
Silica gel H	BAW	.25	.28
Silica gel H	CMW	.0	.0

material used in this study corresponded to the major monosulfate product of synthesis blocked at positions 5 and 6; on the basis of chemical tests this product should have a sulfate at position 3. Reactivity of this compound to agents that oxidize ascorbate and to the ferric chloride test indicated a blocked position 3 on the ascorbate sulfate. Preliminary ^{13}C nuclear magnetic resonance showed the product was not substituted on positions 5 or 6 and indicated probable substitution at position 3. Because structural study is not complete, unambiguous assignment of structure is not possible. On the basis of the experimental evidence given above, the compound is presumed to be ascorbate-3-sulfate.

After addition of synthetic carrier ascorbate-3-sulfate, radioactive material in the S fraction was fractionated by chromatography on a Dowex-1 formate column eluted with a linear gradient system of formate and formic acid as described by Mead and Finamore (*3*). The major radioactive component was doubly labeled with the same ratio of ^{14}C to ^{3}H as the ingested ascorbate. This fraction had a maximum absorbancy at 254 nm in alkaline or neutral solution corresponding to the values given by Mead and Finamore for ascorbate sulfate in brine shrimp cysts. The materials in this formate fraction and the original S fraction were then cochromatographed against chemically synthesized ascorbate-3-sulfate in the systems listed in Table 1. In all cases, correspondence of a positive iodine vapor reaction was observed for this metabolite of ascorbic acid and the synthetic ascorbate-3-sulfate.

Direct column chromatography on DEAE cellulose of both freshly voided urine and the S fraction of urine from normal adult males showed a substance that absorbs ultraviolet light and that is eluted by the sulfuric acid linear gradient at the position corresponding to synthetic ascorbate-3-sulfate. When the S fraction from normal male urine was cochromatographed on DEAE cellulose with ascorbate-3-[^{35}S]sulfate, there was exact coincidence at the position of the ascorbate-3-sulfate between the ^{35}S radioactivity and the urinary material that absorbs ultraviolet light.

Under conditions of high intake of ascorbate in man (100 or more milligrams per day) much ingested ascorbate is excreted unchanged (*2*). The rapid decomposition of free ascorbic acid in urine makes especially difficult the isolation and identification of true urinary metabolites. Only on a highly restricted intake of ascorbate can the essential metabolites of ascorbate be easily observed. Recent experiments with human volunteers indicate that this metabolism of ascorbate is about 3 percent of the body pool per day (*2*). Fractionation of these essential metabolites in human urine by means of the lead acetate precipitation method of carbohydrate chemistry shows that there are a variety of products somewhat comparable to those we have recently observed in ascorbate metabolites excreted by the guinea pig. It seems likely that the metabolites precipitated by lead acetate at *p*H 4 and at *p*H 8 are in part glucuronide derivatives and carboxylic acids formed by oxidative processes. The S fraction corresponds to about 25 percent of the total metabolites, and in it the major radioactive component is ascorbate-3-sulfate.

The demonstration of ascorbate-3-sulfate in human urine and in brine shrimp cysts, as well as our studies which show its existence in guinea pig, rat, and trout urine, suggests that this compound is a fairly ubiquitous metabolite of ascorbate in higher animal systems. The biological role of ascorbate sulfate is of some interest. It could act as a sulfate donor, as has been suggested by Chu and Slaunwhite (*4*) and Mumma (*5*). It could have a function in transport across cellular membranes in as much as ascorbate-3-sulfate is a di-negative ion at physiological *p*H's and appears to interact strongly with metal ions. It could also be part of the ascorbate pool in man and animals. The ascorbate sulfate could also play a part in the transport of ascorbate across the blood-brain barrier and in the concentration of ascorbate in brain tissue. Hammarström and others have demonstrated that free ascorbate does not rapidly cross this barrier (*6*).

This ascorbate sulfate derivative adds one more substance to the growing list of compounds that suggests a greater biochemical role for ascorbate than that of a simple antioxidant. Blaschke and Hertting (*7*) have recently demonstrated 2-methylascorbate in the rat. Earlier, Kiss and Neukom (*8*) have suggested that 2-methylindol ascorbate was the ascorbigen of cabbage. In addition, a variety of 2- and 3-ascorbate derivatives have been prepared by chemical means. The presences of a number of yet unidentified six-carbon metabolites in human and guinea pig urine indicate the complexity of essential ascorbate metabolism.

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References and Notes

1. L. Hellman and J. J. Burns, *J. Biol. Chem.* **230**, 923 (1958); E. M. Baker, J. C. Saari, B. M. Tolbert, *Amer. J. Clin. Nutr.* **19**, 371 (1966).
2. E. M. Baker, R. E. Hodges, J. Hood, H. E. Sauberlich, S. C. March, *Amer. J. Clin. Nutr.* **22**, 549 (1969); ———, J. E. Canham, *ibid.* **24**, 444 (1971).
3. C. G. Mead and F. J. Finamore, *Biochemistry* **8**, 2652 (1969).
4. T. M. Chu and W. R. Slaunwhite, Jr., *Steroids* **12**, 309 (1968).
5. R. O. Mumma, *Biochim. Biophys. Acta* **165**, 517 (1968).
6. L. Hammarström, *Acta Physiol. Scand.* **70** (Suppl. 289), 1 (1966).
7. E. Blaschke and G. Hertting, *Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol.* **266**, 297 (1970).
8. G. Kiss and H. Neukom, *Helv. Chim. Acta* **49**, 989 (1966).

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Leucylglycinamide Released from Oxytocin by Human Uterine Enzyme

Abstract. *Uteri of pregnant and nonpregnant women contain enzymic activities which inactivate oxytocin. A potent enzyme, which has been partially purified from uterine homogenates, cleaves the prolyl–leucyl peptide bond of oxytocin. This finding associates for the first time the release of the dipeptide leucylglycinamide with the degradation of neurohypophyseal hormones.*

Extracts of human uterine tissue inactivate the neurohypophyseal hormone oxytocin (*1*, *2*). To clarify the underlying enzymic reactions, we have identified products of oxytocin digestion. We adapted a fractionation procedure, used previously in studies (*3*) of uterine extract from rats, for the partial purification of uterine extracts from pregnant and nonpregnant women; the former at a time of elective caesarean section. We used strips of the uterine wall of