active fraction crystallized from acetone to give lupeyl cerotate (**9**), with m.p. 84°–85°C; specific rotation −16.1°(0.56 percent in chloroform); infrared bands at 1720 cm^{-1} (ester) and 1640 cm^{-1} (olefin). Its structure was established on the basis of the products obtained after hydrolysis (1*N* KOH, 90 percent ethanol, 5 hours). Lupeol was separated from the potassium salt of the fatty acid by ether extraction, then passed through a short basic alumina column (benzene to chloroform), and crystallized from ethanol, m.p. 216°–217°C. Comparison (as judged by mixture melting point, infrared and nuclear magnetic resonance spectra, and specific rotation) of the isolated lupeol (**10**) with an authentic sample showed them to be identical; their acetates were also identical (*12*). The potassium salt of the fatty acid was suspended in dilute HCl and warmed until globules formed; the mixture was cooled and filtered; the fatty acid was crystallized from benzene and identified as cerotic (hexacosanoic) acid, m.p. 88°–90°C, with infrared bands at 3500 to 2500 cm^{-1} (carboxylic acid OH) and 1705 cm^{-1} (carboxyl). The mass spectrum showed a molecular ion at *m/e* 396.3923 (71 percent) (calculated mass for $C_{26}H_{52}O_2$ is 396.3967 and prominent peaks at *m/e* 339 (4 percent), 297 (9 percent), 241 (7 percent), 185 (13 percent), 129 (36 percent), 73 (63 percent), and 43 (100 percent), a pattern characteristic for an unbranched long-chain fatty acid (*13, 14*).

The aqueous extract residue (B) was examined for its active constituents by a number of procedures including extraction, precipitation, and chromatography; but in the end the separation was accomplished by chromatographing the total acetates (as with fraction G) on silicic acid. Acetylation of 10 g of residue B gave 15 g of acetates which were separated into nine fractions of which the most active yielded 0.64 g of the heptaacetate **1**. The glycoside **2** is present in a larger amount in fraction B, and that isolated from G apparently represents a quantity carried over as a result of a slightly favorable partitioning into chloroform.

The combination of the two new isolated substances, (+)-catechin xyloside (**2**) and lupeyl cerotate (**9**), elicits a feeding response approaching 60 to 70 percent of that obtained from the residue of the initial extract. The difference probably represents the effect of other, unidentified constituents acting additively or synergistically, but these were not readily indicated in our study.

Since *S. multistriatus* is the principal vector in the spread of the Dutch elm disease fungus, *Ceratocystis ulmi* (Buisman) C. Moreau, knowledge related to the chemical factors influencing the feeding behavior of the insect might be of use in measures designed to control this disease.

RAYMOND W. DOSKOTCH
SUJIT K. CHATTERJI
Division of Natural Products Chemistry, College of Pharmacy, Ohio State University, Columbus 43210
JOHN W. PEACOCK
Forest Insect and Disease Laboratory, Northeastern Forest Experiment Station, Forest Service, U.S. Department of Agriculture, Delaware, Ohio 43015

References and Notes

1. For specific examples and references see A. J. Thorsteinson, *Ann. Rev. Entomol.* **5**, 193 (1960); S. D. Beck, *ibid.* **10**, 207 (1965); M. Jacobson, *ibid.* **11**, 403 (1966).
2. S. R. Loschiavo, S. D. Beck, D. M. Norris, *Ann. Entomol. Soc. Amer.* **56**, 764 (1963); J. E. Baker, D. P. Rainey, D. M. Norris, F. M. Strong, *Forest Sci.* **14**, 91 (1968).
3. J. E. Baker and D. M. Norris, *Ann. Entomol. Soc. Amer.* **60**, 1213 (1967).
4. J. W. Peacock, B. H. Kennedy, F. W. Fisk, *ibid.*, p. 480.
5. D. M. Norris and J. E. Baker, *J. Insect Physiol.* **13**, 955 (1967).
6. F. E. King, T. J. King, L. C. Manning, *J. Chem. Soc.* 563 (1957).
7. K. Freudenberg and L. Purrmann, *Ann. Chem.* **437**, 276 (1924). For a summary of the evidence supporting the stereochemistry at C-2 and C-3, see W. B. Whalley, in *The Chemistry of Flavonoid Compounds*, T. A. Geissman, Ed. (Macmillan, New York, 1962), p. 441.
8. A study of the mass spectra of flavans was made by J. W. Clark-Lewis, *Aust. J. Chem.* **21**, 3025 (1968).
9. Developed descendingly on Whatman No. 1 sheets with the solvent systems ethyl acetate, pyridine, water (10 : 4 : 3); ethyl acetate, acetic acid, formic acid, water (9 : 1.5 : 0.5 : 2); and *n*-butanol, acetic acid, water (5 : 1 : 2). The R_f values were 0.28, 0.25, and 0.29, respectively.
10. C. S. Hudson and J. M. Johnson, *J. Amer. Chem. Soc.* **37**, 2748 (1915).
11. K. Biemann, D. C. DeJongh, H. K. Schnoes, *ibid.* **85**, 1763 (1963).
12. I. M. Heilbron, G. L. Moffet, F. S. Spring, *J. Chem. Soc.* **1934**, 1583 (1934). The authentic sample was obtained from *Sweetia panamensis* Benth, as reported by T. J. Fitzgerald, J. L. Beal, J. B. LaPidus, *J. Pharm. Sci.* **52**, 712 (1963).
13. K. Biemann, *Mass Spectrometry Organic Chemical Applications* (McGraw-Hill, New York, 1962), pp. 251–255.
14. Lupeyl cerotate may be the same compound as that reported without any physical constants by Baker and Norris (*3*).
15. Supported by grant No. 1 from the Forest Service, U.S. Department of Agriculture to Ohio State University. We thank Dr. R. L. Foltz of Battelle Memorial Institute for the mass spectra determined on an AEI MS-902 mass spectrometer. The infrared spectra were obtained on a Perkin-Elmer 257 spectrophotometer, ultraviolet spectra on a Cary model 15 instrument, and the optical rotations on a Zeiss polarimeter or a Jasco ORD/UV-5 spectropolarimeter. We thank Mrs. Gail Kleiner for technical assistance. Satisfactory elemental analyses were obtained for all the compounds reported.

30 October 1969

5S RNA Synthesized by Escherichia coli in Presence of Chloramphenicol: Different 5′-Terminal Sequences

Abstract. Escherichia coli *cells, grown in the presence of chloramphenicol, synthesize a low molecular weight RNA (CM-5S RNA) not bound to ribosomes which is similar to ribosomal 5S RNA. Oligonucleotide patterns derived from ribonuclease digests of 5S RNA and of CM-5S RNA are indistinguishable except that the 5′-terminal oligonucleotides differ. Whereas the nucleotide sequence of the 5′-terminus of normal 5S RNA is pUpG-, there are three alternate sequences of the 5′-terminus of CM-5S RNA: pUpUpG-, pUpUpUpG-, and pApUpUpUpG-.*

In the presence of chloramphenicol, *Escherichia coli* cells synthesize an RNA (CM-5*S* RNA) (*1*) of low molecular weight. The CM-5*S* RNA, in contrast to normal ribosomal 5*S* RNA, is not bound to ribosomes (or to "chloramphenicol particles") but is found free in the 100,000*g* supernatant fraction of the centrifuged cell homogenates (*2*). Although CM-5*S* RNA is eluted from Sephadex G-100 columns in the same position as normal 5*S* RNA, it is eluted from methylated albumin kieselguhr (MAK) columns somewhat later than normal 5*S* RNA (*2*). We now report a difference in primary structure between CM-5*S* RNA and normal ribosomal 5*S* RNA.

Normal *Escherichia coli* EA2 cells and those treated with chloramphenicol (100 μg/ml) were grown in a low phosphate medium containing ^{32}P; the RNA was prepared by extraction of the whole cells with phenol at 4°C, in the presence of whole cell, nonradioactive, carrier RNA (1 mg/ml). The RNA was precipitated by alcohol, and then fractionated by Sephadex G-100 gel filtration. The fractions corresponding to 5*S* RNA were collected, precipitated in alcohol, and purified by MAK column chromatography. The purified 5*S* RNA and CM-5*S* RNA were digested by pancreatic ribonuclease and ribonuclease T1 and analyzed by two-dimensional oligonucleotide fractionation (*3*).

The two-dimensional fractionation

patterns of ribonuclease T1 digests of CM-5*S* RNA and normal 5*S* RNA (Fig. 1) are similar except in one area; the CM-5*S* RNA lacks the normal 5′-terminal oligonucleotide pUpGp (spot 18), but contains three additional oligonucleotides which are absent from digests of normal 5*S* RNA (spots 25, 26, and 27). The nucleotide sequences of these three oligonucleotides are: spot 25, pApUpUpUpGp; spot 26, pUpUpGp; and spot 27, pUpUpUpGp. The nucleotide sequences of spots 26 and 27 were determined by alkaline hydrolysis of the oligonucleotides followed by electrophoretic fractionation of the digest on Whatman No. 1 paper at *p*H 3.5 (*3*); spot 26 yielded pUp, Up, and Gp; and spot 27 yielded pUp, (Up,Up), and Gp.

The sequence of spot 25 is based on the following results. (i) Fractionation of alkaline hydrolyzates at *p*H 3.5 yielded products in the positions of Gp and Up only, the latter containing radioactivity (count/min) equivalent to five phosphate residues as compared to the one phosphate residue of Gp. However, the material in the position of Up can be fractionated into two components by descending chromatography with either an isopropanol, water, and concentrated ammonia (70 : 30 : 1) (*3*) system or an ethanol, ammonium acetate (*4*) system; of the two components, one migrates rapidly, coinciding with Up (three phosphate residues) and the other migrates more slowly (two phosphate residues). (ii) Digestion of spot 25, with pancreatic ribonuclease yields three products, Gp, (Up,Up), and a product (product **1**) containing radioactivity (count/min) equivalent to three phosphate residues. Product **1**, on Whatman No. 1 paper (*p*H 3.5), migrates between Up and pGp, and, on DEAE paper (*p*H 1.7), migrates much more slowly than Gp. After being subjected to alkaline hydrolysis and fractionation on Whatman No. 1 paper at *p*H 3.5, product **1** yields only one product which migrates with Up, but which can be fractionated into two components by chromatography as described above: Up, and a slower migrating component containing twice the radioactivity as Up. (iii) Product **1**, after being treated with bacterial alkaline phosphomonoesterase, subjected to alkaline hydrolysis, and fractionated at *p*H 3.5, yielded a single product in the position of Ap. Thus the sequence of product **1** seems to be pApUp and that of spot 25, pApUpUpUpGp. The results of digestion of spot 25 with ribonuclease T2, snake venom phosphodiesterase, and spleen acid ribonuclease support this conclusion.

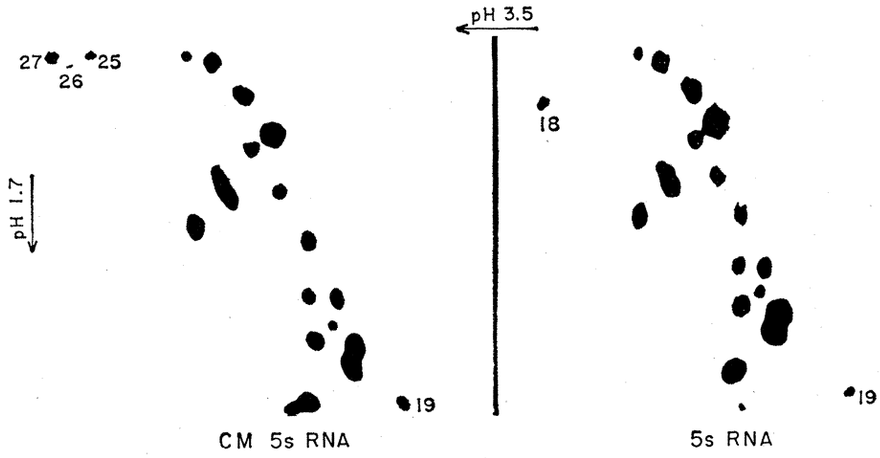

Fig. 1. Radioautograph of two-dimensional fractionations of ribonuclease T1 digests of normal *Escherichia coli* ribosomal 5*S* RNA and 5*S* RNA synthesized by *E. coli* grown in the presence of chloramphenicol (CM-5*S* RNA). Fractionation in the first dimension (right to left) is by electrophoresis (1 hour, 3000 volts) on cellogel strips at *p*H 3.5 (5 percent acetic acid and 0.5 percent pyridine). Fractionation in the second dimension (top to bottom) is by electrophoresis (2½ hours, 1500 volts) on DEAE-cellulose paper at *p*H 1.7 (7 percent formic acid). Spot 19 is CpApU-OH, the 3′-terminal sequence of normal 5*S* RNA; spot 18 is pUpGp, the 5′-terminal sequence of normal 5*S* RNA; spots 25, 26, and 27 are the alternate 5′-terminal sequences of CM-5*S* RNA.

When the molar ratios of oligonucleotides are calculated for CM-5*S* RNA in a manner similar to that described by Brownlee *et al.* for 5*S* RNA (*5*), each of the three additional oligonucleotides of CM-5*S* RNA (spots 25, 26, and 27) is present in a yield substantially less than 1 mole. However, the sum of their yields is 0.8 ± 0.2 mole. This indicates that these three oligonucleotides can be considered as alternate 5′-terminal sequences of an RNA molecule that is approximately 120 base residues in length. The yields of the other oligonucleotides of CM-5*S* RNA are similar to those of the corresponding oligonucleotides from normal 5*S* RNA.

The two-dimensional fractionation patterns of CM-5*S* RNA digested by pancreatic ribonuclease show the same spots present in normal 5*S* RNA (including the 5′-terminal oligonucleotide pUp) plus one additional oligonucleotide which migrates more rapidly than GpUp in the first dimension and somewhat more slowly than GpUp in the second dimension. This oligonucleotide yields analyses similar to those of product **1** (pApUp) obtained by pancreatic ribonuclease digestion of oligonucleotide No. 25, which is obtained in fractionations of ribonuclease T1 digests of CM-5*S* RNA. In the CM-5*S* RNA digested with pancreatic ribonuclease, the yield of pUp is much lower than 1 mole, but the combined yield of pUp and pApUp approaches 1 mole. No other significant differences were detected in the yields of the other oligonucleotides of these two RNA's digested with pancreatic ribonuclease.

These results lead us to conclude that CM-5*S* RNA contains three major alternate 5′-terminal sequences longer than the 5′-terminal sequence of normal 5*S* RNA, pUpG- (enclosed by broken line).

1 (spot 25)	pApUpU	pUpG-
2 (spot 27)	pUpU	pUpG-
3 (spot 26)	pU	pUpG-

The presence of these three sequences is consistent with the hypothesis that CM-5*S* RNA is a precursor of normal 5*S* RNA. The two shorter sequences (spots 26 and 27) may be independent transcription products or could conceivably be derived, by specific cleavages, from the longer sequence, pApUpUpUpGp (spot 25). The 5*S* RNA may be first synthesized as an RNA chain slightly longer than the mature ribosomal 5*S* RNA; the additional 5′-terminal base residues of this chain would be cleaved from the sequence when the precursor RNA chain of 5*S* RNA is integrated into the ribosome.

This type of cleavage could conceivably be accomplished by the potassium-activated phosphodiesterase of *E. coli*, ribonuclease II (*6*), primarily a ribosome-bound exonuclease which releases 5′-nucleotides from long RNA

chains (*6*). The cleavage would occur early during biosynthesis of the 50*S* ribosomal subunit since the 5*S* RNA contained in 43*S* ribosomal precursor particles has the normal 5′-terminal sequence pUpG- (*7*).

We do not believe that CM-5*S* RNA is an artifact caused by the presence of chloramphenicol because, when *E. coli* EA2 (an RC^{rel} strain of *E. coli* requiring methionine for normal growth) is grown in the absence of its required amino acid (a situation where RNA synthesis continues despite inhibition of protein and ribosome synthesis), the 5*S* RNA synthesized is modified at its 5′-terminus in the same fashion as CM-5*S* RNA (*8*). Furthermore, an RNA with the same 5′-terminal sequences as CM-5*S* RNA can be isolated from normally growing *E. coli* cells pulse-labeled during the exponential phase of growth (*8*). The conversion of 5*S* RNA to the form terminated in pUpG appears to occur in ribosomes of normal cells but not in those where protein synthesis, and subsequently ribosome synthesis, has been blocked by amino acid deficiencies or by the antibiotic chloramphenicol.

BERNARD G. FORGET*
BERTRAND JORDAN

Centre de Biochimie et de Biologie Moléculaire, Centre National de la Recherche Scientifique, Marseille, France

References and Notes

1. Abbreviations: RNA, ribonucleic acid; A, adenosine; G, guanosine; U, uridine; p indicates a phosphate group, on the left side a 5′-phosphate and on the right side a 3′-phosphate; DEAE, diethylaminoethyl; and MAK, methylated albumin kieselguhr.
2. F. Galibert, J. C. Lelong, C. J. Larsen, *C. R. Hebd. Seances Acad. Sci. Paris* **265**, 279 (1967).
3. F. Sanger, G. G. Brownlee, B. G. Barrell, *J. Mol. Biol.* **13**, 373 (1965); B. G. Forget and S. M. Weissman, *J. Biol. Chem.* **243**, 5709 (1968).
4. A. C. Paladini and L. F. Leloir, *Biochem. J.* **51**, 426 (1952).
5. G. G. Brownlee and F. Sanger, *J. Mol. Biol.* **23**, 337 (1967); G. G. Brownlee, F. Sanger, B. G. Barrell, *ibid.* **34**, 379 (1968).
6. P. F. Spahr, *J. Biol. Chem.* **239**, 3716 (1964); M. F. Singer and G. Tolbert, *Biochemistry* **4**, 1319 (1965).
7. B. G. Forget and F. Varricchio, *J. Mol. Biol.*, in press.
8. B. Jordan, B. G. Forget, J. Feunteun, R. Monier, in preparation.
9. We thank Prof. R. Monier for many helpful discussions and for making facilities available for this work; we thank Dr. S. M. Weissman and Dr. G. G. Brownlee for useful discussions and for performing certain oligonucleotide analyses. Supported in part by grants 1-F3-GM-38,781 and 1 TO1 AM05581 of the USPHS and by grants from the Délégation Générale à la Recherche Scientifique et Technique and from the CEA (France).
* Present address: Hematology Research Laboratory, Children's Hospital Medical Center, Boston, Massachusetts 02115.

13 November 1969

Floral Inducing Extract from Xanthium

Abstract. *Flower formation was initiated in* Lemna *by acetone extracts of flowering cocklebur* (Xanthium). *These extracts also initiated flower formation in* Xanthium *when they were supplemented with gibberellic acid. No flower formation was initiated in duckweed* (Lemna) *or* Xanthium *by extracts from vegetative* Xanthium. *Extracts from vegetative plants supplemented with gibberellic acid did not initiate flowers in* Xanthium *or* Lemna.

Various claims have been made of isolating substances that will induce flowering in short-day plants (*1*). Lincoln *et al.* (*2*) reported that extracts from several species will induce flowering in vegetative cocklebur *Xanthium*. Although Carr (*3*) has repeated this work, the results have not been fully accepted (*4*) for the following reasons: (i) a relatively small percentage of test plants was induced to flower; (ii) the response of test plants to extracts from vegetative plants was not thoroughly documented (*5*); and (iii) the extracts were tested on one species. We now present evidence for a marked floral inducing activity of extracts from flowering plants and for a lack of floral inducing activity of extracts from vegetative plants of *Xanthium*.

Plants of *Xanthium strumarium* L. (*6*) grown from seeds that were gathered in the vicinity of Chicago, Illinois, served as the source of the extracts. The vegetative plants were grown in 20-hour photoperiods, the natural day-length being supplemented with incandescent light that produced 250 lux at bench height. The flowering plants were given 15 hours of darkness daily.

Twenty extracts were prepared (*7*), ten from flowering plants and ten from vegetative plants. Five hundred grams of expanding leaves and buds were cut from the stem, frozen immediately in liquid nitrogen, crushed into small fragments, and leached with 4 liters of cold acetone. The debris was washed twice with 3 liters of cold acetone (70 percent by volume). One liter of 0.1*N* NH_4OH was added to the combined filtrates, and this solution was reduced in volume by vacuum distillation below 30°C in a circulating evaporator. The resultant extracts (4.5 g) were kept frozen until they were tested.

In the *Xanthium* bioassay, extracts were tested for activity on vegetative plants that had four to six fully developed leaves. At the time of treatment, all leaves and axillary buds below the two youngest fully expanded leaves were trimmed from the plants. With the stem-flap method (*8*), we applied 10 ml of distilled water containing 1 mg of extract, or 1 mg of extract and 0.1 mg of gibberellic acid (GA), or 0.1 mg of GA directly to the vascular tissue of each test plant. All plants were kept in noninductive conditions and were

Table 1. Extract activity evaluated by the *Xanthium* bioassay. One hundred plants were used in each of the six treatments. Ten plants were used to evaluate each of the 20 extracts from flowering or vegetative plants. The figures represent the total number of plants in each stage of flowering (*9*). Plants in stage 2 or above are considered to be flowering. GA, gibberellic acid.

Treatment, extract or chemical	Flowering stage				
	0	1	2–5	6–10	>10
Flowering plants	91	9	0	0	0
Flowering plants + GA	11	13	44	21	11
Vegetative plants	97	3	0	0	0
Vegetative plants + GA	84	16	0	0	0
Gibberellic acid	88	12	0	0	0
Water	100	0	0	0	0

Table 2. Extract activity evaluated by the *Lemna* bioassay. A total of 30,000 plants were used to evaluate the activity of the 20 extracts. The extracts were tested with and without the addition of gibberellic acid (GA). Each figure represents the average response of five lots of 100 plants each.

Treatment	Plants flowering (%)									
Flowering plants	45	31	54	37	58	46	45	51	39	60
Flowering plants + GA	0	0	0	0	0	0	0	0	0	0
Vegetative plants	0	0	9	0	7	0	0	0	2	0
Vegetative plants + GA	0	0	0	0	0	0	0	0	0	0
Gibberellic acid	0	0	0	0	0	0	0	0	0	0
Water	0	0	5	0	11	3	0	0	1	5