Results. At an air temperature of 2 °C, the bees produced heat at a rate dependent upon the number of bees in the group. For a group size of 4252 bees (608 g), the heat production was 3.78 W (0.90 cal/sec) for a constantly maintained cluster core temperature of 34 °C. The average total heat conductance through the bees and comb amounted to 0.028 cal/sec °C, a value which compares with the insulating values of birds and mammals[2]. The entire cluster volume was about 1.686 cm^3 for this group. The theoretical minimum volume of 617 cm^3 for the 4252 bees packed as tightly as possible in a sphere was calculated from volume measurements (acetone displacement of individual bees). The minimum spherical diameter was 10.5 cm, and minimum surface area 353 cm^2. The actual volume was about 2¾ times greater than the theoretical minimum. This is due to the intrusion of wax combs and the resulting nonspherical shape assumed by the bees. The bees occupied the spaces inbetween 3 wax combs, and packed themselves into oval shaped masses of varying thickness depending on the distance between the combs. They formed tight layers on the combs trapping air in each cell. The combs interrupt the cluster, however they also can provide insulation. The temperature on one side of an empty comb (i.e., without honey, pollen or brood) adjacent to the bees was maintained at 33.0 °C, while on the other side of the comb where there were no bees (2 cm separation), the temperature was only 8.3 °C (at an air temperature of 2 °C). This yields an average thermal conductivity of only 0.065×10^{-3} cal/sec cm °C for the comb when covered with the tightly packed layer of living bees[3]. Thermal conductivity figures from the direct measurements are higher ($0.36–0.44 \times 10^{-3}$ cal/sec cm °C). However these values represent only the comb alone without the bees actively forming a tight layer, trapping the air.

Of the total cluster surface area of 934 cm^2, 353 cm^2 were exposed to the cold air inbetween the frames, and an additional 581 cm^2 were adjacent to the insulating combs on both sides of the cluster. Although the total surface area was some 2½ times greater than the theoretical minimum, only 32% of the surface (identical to the theoretical minimum) is actually exposed to the cold air temperatures while the remaining 68% of the surface is adjacent to the insulating combs.

The effectiveness of the comb is clearly seen when bees without comb are examined. With no comb, the same mass and volume of bees would require 39% more heat to maintain the same temperature differential. Insulation by the wax combs is provided by the combined effect of combs and behavioral response to low temperatures by the bees.

Further experiments on thermal relations at cold temperature by bees and a more detailed discussion will be published elsewhere.

1 Southwick, E. E., Comp. Biochem. Physiol. *71A* (1982) 277.
2 Herreid, C. F. II, and Kessel, B., Comp. Biochem. Physiol. *21* (1967) 405.
3 Precht, H., Christophersen, J., Hensel, H., and Larcher, W., Temperature and life. Springer, Berlin 1973.

0014-4754/85/111486-02$1.50 + 0.20/0

Marine indoles of novel substitution pattern from the acorn worm *Glossobalanus* sp.[1]

T. Higa[2], T. Ichiba[2] and R. K. Okuda[3]

Department of Marine Sciences, University of the Ryukyus, Nishihara, Okinawa 903-01 (Japan), and Department of Chemistry, Columbia University, New York (New York 10027, USA), 5 September 1984

Summary. 4,6-Dibromoindole and 4,6-dibromo-2-methylindole have been isolated from the acorn worm *Glossobalanus* sp. The biosynthetic implications of this finding are discussed.

Key words. Marine brominated indoles; biogenesis; antibiotics.

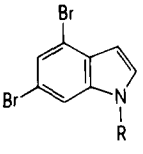
In previous work[4] on the acorn worm *Glossobalanus* sp. (class Enteropneusta, phylum Hemichordata) one of us detected by mass spectrometry the presence of a dibromoindole and a methyldibromoindole in the acetone extract. The amount of material available was limited, which precluded the isolation of these metabolites at that time. When a larger quantity of the organism had been obtained we were able to isolate and characterize the compounds as 4,6-dibromoindole (**1**) and 4,6-dibromo-2-methylindole (**2**), which belong to a novel substitution class of halogenated marine indoles.

Specimens of the animal (5 kg including ingested sand) accumulated in several collections at Miibaru Beach, Okinawa, in March and April, 1983 were thoroughly extracted with acetone in a blender. The extract was concentrated and successively extracted with hexane and ethyl acetate (EtOAc) to yield 10.5 and 3.3 g of oily residues, respectively. The latter oil was chromatographed on silica gel by eluting with hexane and increasing amounts of EtOAc. Of the many fractions exhibiting antibacterial activity[5], fractions eluted with 5:1 hexane-EtOAc were subjected to two more separations on a LiChroprep Si 60 column (3:2 hexane-$CHCl_3$) to give 21 mg of 4,6-dibromoindole (**1**) as a colorless oil and 35 mg of 4,6-dibromo-2-methylindole (**2**) as colorless crystals, m.p. 96–96.5 °C.

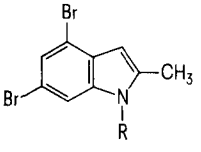
Compound **1**, $C_8H_5Br_2N$, showed EIMS at m/z 277, 275, 273 (M^+) and ^1H NMR ($CDCl_3$) δ6.56 (1H, t, 3-H), 7.21 (1H, t, 2-H), 7.42 (1H, d, 5-H), 7.47 (1H, d, 7-H), and 8.28 (1H, br s, N-H).

Compound **1** was unstable. Treatment of **1** with hot acetic anhydride and pyridine gave the acetyl derivative **3**[6]. ^1H NMR ($CDCl_3$) of **3** contained signals at δ2.59 (3H, s, $COCH_3$), 6.65 (1H, dd, J = 3.6, 0.7 Hz, 3-H), 7.42 (1H, d, J = 3.6 Hz, 2-H), 7.56 (1H, d, J = 1.5 Hz, 5-H), and 8.60 (1H, dd, J = 1.5, 0.7 Hz, 7-H). The low-field shifts of the 2-H and 7-H comparing to the chemical shifts of the parent compound **1**, the observed coupling constant between 2-H and 3-H, and the long range coupling[7] between 3-H and 7-H enabled us to locate unambiguously two bromine atoms at the 4- and 6-positions.

Compound **2**, $C_9H_7Br_2N$, exhibited EIMS at m/z 291, 289, 287 (M^+) and ^1H NMR ($CDCl_3$) δ2.40 (3H, s, 2-CH_3), 6.22 (1H, br s, 3-H), 7.31 (1H, br s, 7-H), 7.35 (1H, d, J = 1.5 Hz, 5-H), and 7.95 (1H, br s, N-H). Compound **2** was similarly acetylated to give a 1-acetyl derivative **4**[8] which showed ^1H NMR ($CDCl_3$) δ2.60 (3H, s, 2-CH_3), 2.66 (3H, s, $COCH_3$), 6.40 (1H, br s, 3-H), 7.52 (1H, d, J = 1.5 Hz, 5-H), and 8.23 (1H, dd, J = 1.5, 0.7 Hz, 7-H). These resonances clearly showed that the 2-, 4-, and 6-positions



1, R = H
3, R = Ac



2, R = H
4, R = Ac

of indole are substituted with the three substituents (2Br, CH_3). Irradiation of the methyl signal at δ2.60 sharpened the signal at δ6.40, indicating that the methyl group was located in the 2-position. Thus, compound **2** is 4,6-dibromo-2-methylindole.

Bromine substitution at the 4- and 6-positions without any substituents at the 3-position has never been reported before from natural sources. To date more than 70 indolic compounds have been isolated from marine organisms[9]. Many of them bear halogens, most probably one of the halogens at the 3-position unless the site is occupied by other substituents. This is consistent with a biosynthetic mechanism involving the attack of peroxidase-mediated cationic species on an indolic substrate, since the 3-position of indole is the site most susceptible to the attack of electrophilic halogenating reagents[10]. Biosynthesis of 3-unsubstituted haloindoles may therefore involve a different mechanistic scheme from that of 3-halogenated indoles. In the biosynthesis of the present compounds **1** and **2** and of the previously-known 5,7-dibromo-6-methoxyindole (**5**)[11] halogenation of an aromatic amino acid such as phenylalanine (tyrosine in case of **5**) may be one of the first steps, and be followed by cyclization to an indole-2-carboxylic acid as proposed earlier[4]. This, however, is contrary to the known biosynthetic scheme for some brominated phenols from aromatic amino acids in algae, in that bromination occurs only at the later stages of the catabolism[12].

1 We wish to thank Prof. Paul J. Scheuer, University of Hawaii for his encouragement and for use of his facilities during spectroscopic work. T.H. gratefully acknowledges the giving of a travel grant by the University of the Ryukyus Foundation for International Exchange.
2 University of the Ryukyus.
3 Columbia University.
4 Higa, T., Fujiyama, T., and Scheuer, P.J., Comp. Biochem. Physiol. *65B* (1980) 525.
5 The fractions were active against *Escherichia coli, Staphylococcus aureus* and *Bacillus subtilis*.
6 M.p. 102–104 °C; HRMS m/z 318.8890 (Calcd for $C_{10}H_7{}^{81}Br_2NO$: 318.8855); IR (KBr) 1705, 1405 1320 cm^{-1}.
7 Batterham, T.J., NMR Spectra of Simple Heterocycles, p. 250. Wiley-Interscience, New York 1973.
8 M.p. 141–143.5 °C; HRMS m/z 331.9060 (Calcd for $^{12}C_{10}{}^{13}CH_9{}^{79}Br^{81}BrNO$: 331.9063); IR (KBr) 1700, 1400, 1370, 1290 cm^{-1}.
9 Christophersen, C., Marine Natural Products: Chemical and Biological Perspectives, vol. 5, p. 295. Ed. P.J. Scheuer. Academic Press, New York 1983.
10 Powers, J.C., Indoles, part 2, p. 137. Ed. W.J. Houlihan. Wiley-Interscience, New York 1972.
11 Higa, T., and Scheuer P.J., Heterocycles *4* (1976) 227.
12 Barrow, K.D., in: Marine Natural Products: Chemical and Biological Perspectives, vol. 5, p. 51. Ed. P.J. Scheuer. Academic Press, New York 1983.

0014-4754/85/111487-02$1.50 + 0.20/0

An economical large scale procedure to purify *E. coli* amplifiable plasmids for DNA sequencing, in vitro transcription and in vitro mutagenesis[1]

Guang-jer Wu[2] and R.E. Cannon

Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta (Georgia 30322, USA), 27 July 1984

Summary. A reproducible and economical procedure for obtaining a large and quantitative yield of highly purified covalently closed circular plasmid DNA is described. The procedure departs in several ways from more commonly used methods. These are a) avoidance of the use of CsCl, ethidium bromide and ultracentrifuge, b) enrichment of the plasmid DNA by selective denaturation of chromosomal DNA with an alkaline-SDS solution, c) enrichment of covalently closed circular plasmid DNA by extraction with acid-phenol, and d) removal of small degraded RNA fragements by molecular sieve chromatography after digestion with RNase A. The plasmid DNA prepared by this new procedure is free of contaminants and has been used for DNA sequencing, in vitro transcription, transformation and in vitro mutagenesis.
Key words. Economy large scale purification; CCC plasmid DNA; no EtBr; no ultracentrifugation.

The importance of plasmid DNA to recombinant DNA technology has led to the development of various methods for isolating plasmid DNA. Most of the small scale methods are useful for rapid screening of a large number of colonies, but are not practical for preparation of large quantities of pure covalently closed circular plasmid DNA. Several large scale procedures[3–8] have been developed. Each has its advantages, but all suffer from at least one major disadvantage, such as reproducibility, ability to achieve truly large quantities of plasmids, and/or the need for expensive chemicals and equipment. For these reasons, we have developed an economical and useful large scale method for the preparation of pure covalently closed circular plasmid DNA which avoids most of the disavantages of presently available procedures. The method avoids exposure of DNA to ethidium bromide and does not require the use of expensive CsCl and ultracentrifuge. Plasmid DNAs were recovered in good yield with high purity and biological activities.

Procedure and results. The *E. coli* K12 strain HB101 (pro$^-$, leu$^-$, thi$^-$, lacy$^-$, hsdR$^-$, endA$^-$, recA$^-$, rpsL20, ara-14, galK2, Xyl-5, mtl-1, supE44) was used as the host bacterial strain for all plasmids. The recombinant plasmid used in this study was pAd123 which contained an insert of 1.65 Kb of an adenovirus type 2 DNA fragment (31.5 to 36.9% map unit). This fragment was derived by a double digestion of adenovirus type 2 DNA with restriction enzymes Hind III and Sal 1. Fifty other pBR322 derived recombinant plasmids with inserts ranging from 0.6 to 8 Kb were also used.

All procedures are expressed per liter of harvested bacterial cells and can be easily scaled up to any desired volume. Bacteria with plasmids were grown in 1 l of M9CA medium[9] containing 50 μg/ml of ampicillin (Sigma) until an absorbancy of 0.7 to 1.0 at 550 nm was obtained. Chloramphenicol (Sigma) was then added to a final concentration of 140 μg/ml and the culture was continued for another 16 h to allow amplification of the plasmid[10]. For preparation of plasmids alkaline-SDS-denaturation[11] was chosen as the first step to effectively remove most of the chromosomal DNA and cellular RNA; the enriched plasmid DNA was recovered in the supernatant. To accomplish this, the cells were harvested by centrifugation at 4000 × g for 10 min at 4 °C. The cell pellet was gently suspended in 20 ml of a freshly prepared lysozyme solution [2 mg/ml lysozyme (Sigma), 9 mg/ml D-glucose, 10 mM Na·EDTA (pH 7.9) and 25 mM Tris·HCl (pH