

IN VITRO CULTURE OF RABBIT EMBRYOS

V. H. RAO, K. P. AGARWAL, I. V. MONGHA* AND N. K. BHATTACHARYYA

Division of Physiology and Climatology, *Division of Experimental Medicine and Surgery, Indian Veterinary Research Institute, Izatnagar 243122, India.

In vitro culture of rabbit embryo is believed to require a gaseous environment containing 5% carbon dioxide in air¹. The object of the present experiment was to test if air sealed in a test tube under atmospheric pressure can support the *in vitro* development of rabbit embryos. Individually caged eight albino does and two bucks were used in the experiment. Six does (donors) were mated with a fertile buck and 48 hr later the embryos were collected². Simultaneously two recipient does were mated with a vasectomized buck to induce pseudopregnancy. The embryos were washed twice with culture medium-phosphate buffered saline³, with heat-treated goat serum (1:1). Twenty morphologically normal embryos were placed in 0.5 ml of culture medium at 37°C contained in 2.5 ml capacity air tight screw cap tubes. The tubes with tightened caps were placed immediately in an incubator maintained at 37 ± 1°C. After 24 hr the embryos were recovered from the culture tubes, examined under a microscope, and seven of them were transferred into the fallopian tubes² of recipient does to test their viability.

At the end of the 24 hr culture period, all the 20 embryos underwent atleast one cleavage division (table 1). One of the two recipient does gave birth to a normal male bunny after 28 days of transfer.

Since all the embryos underwent atleast one cleavage division, rabbit embryos do get the necessary amount of carbon dioxide atmosphere in this culture system at least for a short term *in vitro* development.

TABLE I

Development stage of embryos

At collection	After 24 hr culture
8-cell (13)	16-cell (13)
12-cell (4)	Early morulae (4)
16-cell (3)	Early morulae (3)

Figures in parentheses indicate number of embryos.

The authors thank the Director of the Institute for facilities.

20 November 1981

1. Maurer, R. R., In *Methods in mammalian reproduction* (ed.) J. C. Daniel, New York, Academic Press, 1978, p. 259.
2. Agarwal, K. P., Mongha, I. V. and Bhattacharyya, N. K., *Indian J. Exp. Biol.*, 1979, **70**, 1314.
3. Whittingham, D. G., *Nature (London)* 1971, **233**, 125.

**A NOVEL PALLADIUM(II)
ACETYLACETONATO MIXED LIGAND
COMPLEX CONTAINING
HEXAFLUOROACETYLACETONATE AS
COUNTER ION**
B. K. SAHU, S. B. MISRA AND B. K. MOHAPATRA
Department of Chemistry,
B. J. B. College, Bhubaneswar 751014, India

THE acetylacetonate moiety (ac.ac) in terminal carbon bonded complex [Pd(bpy)Cl(ac.ac)]¹ has still an acidic proton and the acetylacetonate molecule can coordinate to another metal ion to give bi- and trinuclear complexes containing bridging ac.ac ligand². Also, the ionic γ -carbon bonded acetylacetonate complex K [Pt(ac.ac) Cl (γ -ac.ac)] reacts with divalent metal ions to produce compounds of the type: [(ac.ac).Cl.pt (γ -ac.ac) M(II) (γ -ac.ac)Pt.Cl (ac.ac.)]^{3,4}. On the other hand, the neutral palladium(II) complex of the type [Pd(ac.ac) (γ -ac.ac)Py]⁵ (A) contains the central carbon bonded acetylacetonate molecule in the keto form. Such a compound can have ketonic coordination with other metal ions or form O,O'-chelate if the proton at the γ -carbon atom can be abstracted. We have been studying the reaction of a similar complex [Pd(ac.ac) (γ -ac.ac) (γ -pic)] with a number of substitution labile complexes such as Cu(ac.ac)₂, Pd(ac.ac)₂, Be(ac.ac)₂, Pd(hfac)₂, [Pd(hfac) (bpy)] hfac and the acetates and chlorides of Zn, Cu, Ni, etc. in different solvent media. It was found that the substitution labile complexes either did not react or react under rigorous conditions (such as refluxing for a long time) resulting in the cleavage of the Pd-C bond. But the reaction of [Pd(hfac) (bpy)] hfac resulted in isolation of a new complex of the composition [Pd(ac.ac) (bpy)] hfac.

[Pd(ac.ac) (γ -ac.ac) (γ -pico)] (0.25 g) was dissolved in dichloromethane (5 ml) and added to an acetone solution (10 ml) of [Pd(hfac) (bpy)] hfac (0.42 g). The resulting solution was stirred at ambient temperature. After 20 min, light yellow precipitate

*hexa fluoroacetyl acetate

appeared and the solution was stirred for an additional 5 hr. The precipitate was filtered and recrystallised from acetone-petroleum ether.

C, H, N were determined by microanalysis [Pd(ac.ac) (bpy)] hfac. Found: C, 42.32; H, 2.82; N, 4.94; Calcd. C, 43.23; H, 2.82; N, 4.92. Molecular weight was determined in acetone solution by cryoscopic method. Found: 551, Calcd. 558.2. Infrared spectra were recorded in nujol mulls on JASCO I.R. E(4000–600 cm^{-1}) and Hitachi EPI-L (700–200 cm^{-1}) spectrometers. A JEOL-MHZ-100 spectrometer was used to obtain ^1H NMR spectra in CD_3COCD_3 containing tetramethyl silane as an internal reference.

From the analysis, the complex has the composition [Pd(ac.ac) (bpy)] hfac. Comparison of the infrared spectra of this compound with that of the starting materials made the assignments easier. $\gamma(\text{C}=\text{O}) + \gamma(\text{C}=\text{C})$ of the O-bonded ac.ac chelate appeared $\approx 1500\text{--}1600\text{ cm}^{-1}$, $\gamma(\text{C}=\text{O})$ due to ionic hfac was observed around 1670 cm^{-1} and though there are several bands due to coordinated bipyridine, the characteristic band due to pyridine ring vibration was found at 1615 cm^{-1} in the present complex. Observation of $\gamma(\text{Pd}-\text{O})$ and $\gamma(\text{Pd}-\text{N})$ at 475 and 335 cm^{-1} respectively further substantiated the formulation of the complex.

In the ^1H NMR spectra, methine proton of ac.ac resonates at 5.66 and that of hfac at 5.88 ppm, methyl protons of ac.ac resonate at 2.14 and 2.24 ppm and bipyridine ring protons at 8.2, 8.8 and 9.0 ppm. The intensity ratios are 1:3:4 respectively for methine, methyl and bipyridine protons. Hence the NMR spectral data corroborate the infrared evidence for the nature of the complex. Probably the carbon bonded ac.ac. replaces the hfac chelate to form an intermediate complex (B). The Pd-C bond is then attacked by hfac anion in an attempt to abstract the proton at the γ -carbon atom as a result of which the Pd-C bond breaks and compound C' is produced.

27 April 1981

SOME MESOZOIC PLANT BEDS FROM THE HIGHER HIMALAYA OF BHUTAN

T. M. GANESAN, R. K. CHATURVEDI AND K. P. REDDY

Geological Survey of India, Nilofar, Rajbagh, Srinagar 190008, India.

THE higher Himalaya of Bhutan forms a WNW-ESE trending synclorium, flanked to its south and east by the older crystalline rocks, while to the north and southwest lie respectively the Tethyan sedimentaries of the Tsang Po valley and Phari basin of Tibet.

The rocks of the Lingshi Group, which range in age from Jurassic to Cretaceous, unconformably overlie the Shodug Formation of Permian age. The stratigraphic sequence of the Lingshi Group is given in table 1.

The Mo Chu Formation forming the lower part of Lingshi Group comprises a 55 m carbonaceous slate with minor quartz arenite in the upper part. A newly discovered Mesozoic flora from the Upper part of the Mo Chu Formation at a locality about 4 kilometres to the north of Yale La along the course of the Mo Chu river is reported here.

The assemblage comprises species of *Cladophlebis*, *Sphenopteris*, *Pachypteris* sp. cf. *P. indica*, *Ptilophyllum* sp. cf. *P. acutifolium*, *Elatocladus* sp., *Pagiophyllum* sp. and *Conifero-caulon* sp. cf. *C. rajmahalense*. Besides these, there are other fragmentary plant remains which are yet to be identified.

The assemblage is dominated by *Elatocladus*,

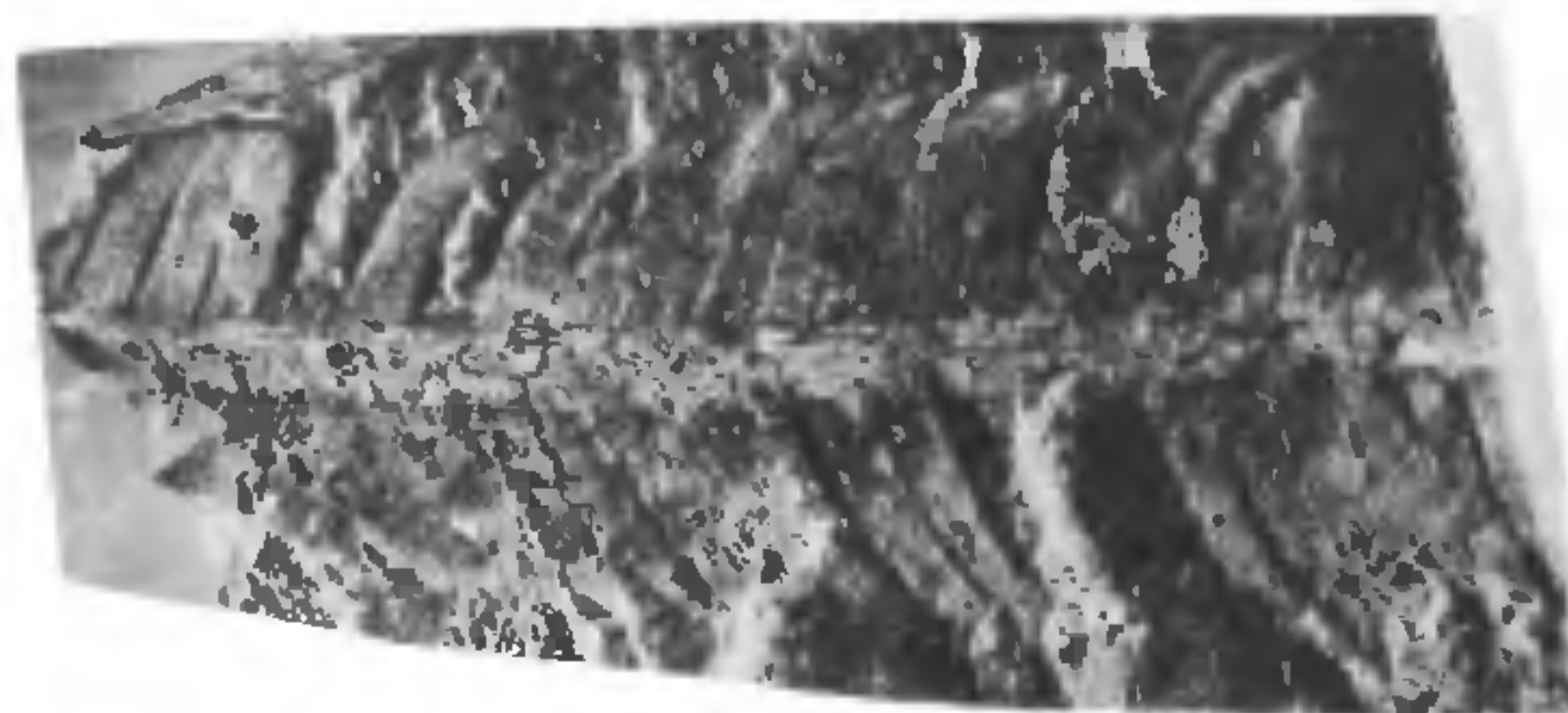


Figure 1. *Ptilophyllum* sp. cf. *P. acutifolium* $\times 1.6$.

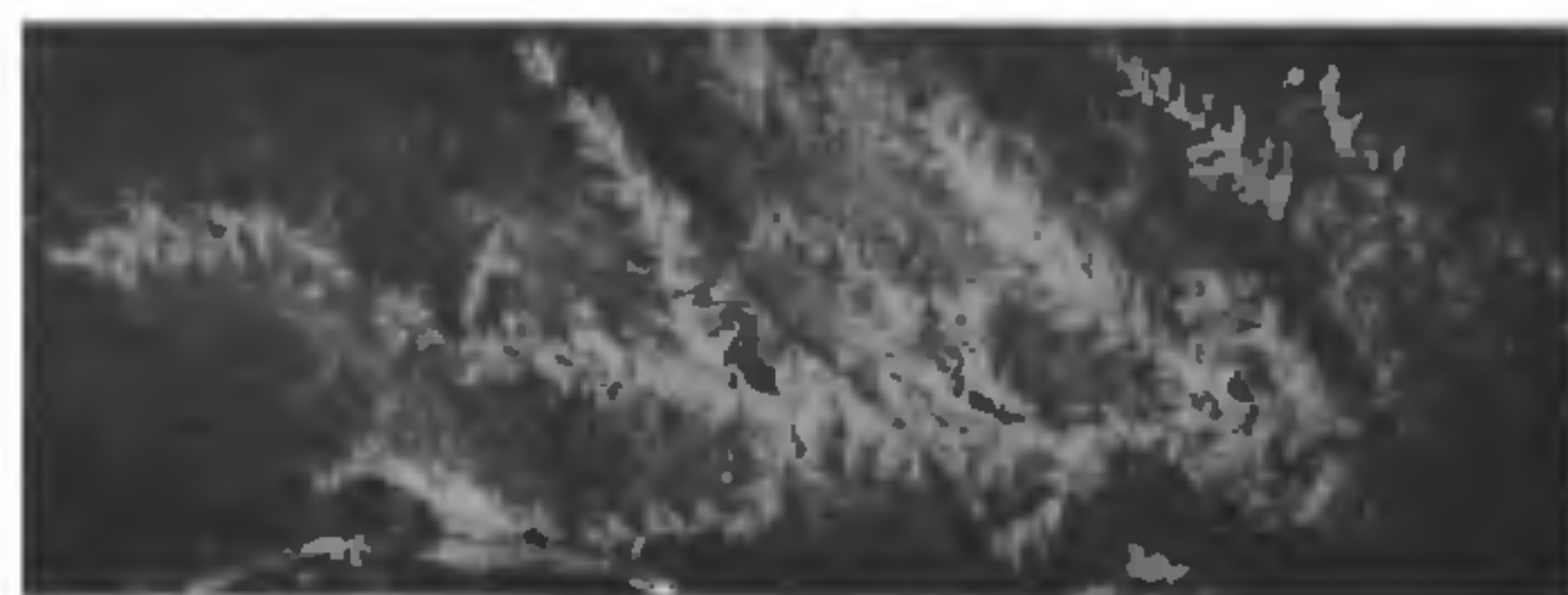


Figure 2. *Pagiophyllum* sp. $\times 1.1$

1. Kanda, Z., Nakamura, Y. and Kawaguchi, S., *Inorg. chem.*, 1978, 17, 910.
2. Yanse, N., Nakamura, Y. and Kawaguchi, S., *Inorg. chem.*, 1978, 17, 2874.
3. Lewis, J. and Oldham, C., *J. Chem. Soc.*, 1965, 6740.
4. Nakamura, Y. and Nakamoto, K., *Inorg.chem.*, 1975, 14, 63.
5. Baba, S., Ogura, T. and Kawaguchi, S., *Bull. Chem. Soc.*, Jpn, 1974, 47, 665.