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O(2),1,9-TRIMETHYLURIC ACID AND 1,3,7,9-TETRAMETHYLURIC ACID IN LEAVES OF DIFFERENT *COFFEA* SPECIES

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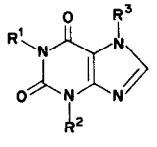
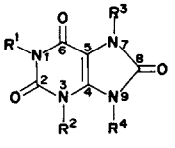
Key Word Index—*Coffea*; Rubiaceae; leaves; 2-methoxy-1,9-dimethyl-7.9-dihydro-1H-purine-6,8-dione; 1,3,7,9-tetramethyl-7,9-dihydro-1H-purine-2,6,8(3H)-trione.

Abstract—*O*(2),1,9-Trimethyluric acid and 1,3,7,9-tetramethyluric acid were isolated from young leaves of *Coffea liberica*, *C. arnoldiana*, *C. dewevrei* var. *excelsa* and var. *aruwimiensis*. The first purine has not been found before in nature; its identification required the synthesis of nearly all of its possible isomers.

INTRODUCTION

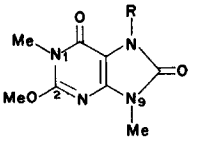
Plant genera containing methylated xanthines belong to widely separated families and even within such genera qualitative and quantitative differences exist. Since the investigations of Bertrand [1] in 1905 it is known that the *Coffea* species of Madagascar and its neighbouring islands either lack or have almost no caffeine (1,3,7-trimethylxanthine) (**1**). Since many *Coffea* species have not yet been investigated chemically, we examined 26 species collected in Africa, Madagascar, and Indonesia.



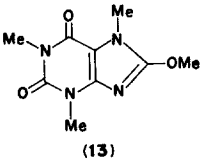


Caffeine (**1**) $R^1 = R^2 = R^3 = Me$
(**6**) $R^1 = Me$; $R^2 = R^3 = H$
(**7**) $R^1 = R^3 = H$; $R^2 = Me$
(**8**) $R^1 = R^2 = H$; $R^3 = Me$

(**3**) $R^1 = R^2 = R^3 = R^4 = Me$
(**4**) $R^1 = R^2 = R^3 = R^4 = H$
(**5**) $R^1 = Me$; $R^2 = R^3 = R^4 = H$
(**9**) $R^1 = R^4 = Me$; $R^2 = R^3 = H$
(**10**) $R^1 = R^4 = Me$; $R^2 = H$; $R^3 = COMe$
(**11**) $R^1 = R^2 = R^4 = Me$; $R^3 = H$



(**2**) R = H
(**12**) R = Me



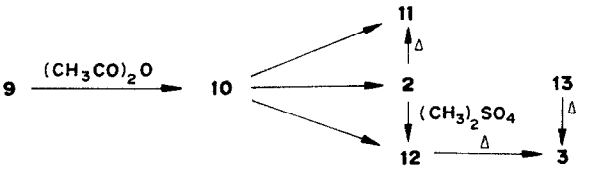
(**13**)

RESULTS AND DISCUSSION

We isolated two substances not detected before in any *Coffea* species, namely 2-methoxy-1,9-dimethyl-7,9-dihydro-1H-purine-6,8-dione (*O*(2),1,9-trimethyluric acid) (**2**) and 1,3,7,9-tetramethyl-7,9-dihydro-1H-purine-2,6,8(3H)-trione (1,3,7,9-tetramethyluric acid) (**3**). They were found together in young leaves of *Coffea liberica* Bull ex Hiern, *C. arnoldiana* De Wild., *C. dewevrei* De Wild. et Durand var. *excelsa* Chev. and *C. dewevrei* De Wild. et Durand var. *aruwimiensis* (De Wild.) Chev. The concentrations of the two purines varied between 0·01 and 2% dry wt, depending on plant age. Both substances were also found in the fruits (pericarp as well as seeds) of *C. arnoldiana* at

a concentration of *ca* 0·1% (dry wt). We were unable to detect either of the two purines in the leaf material of 23 other *Coffea* species collected in Madagascar, although 0·3 g of each sample was worked up, giving a detection limit of 0·001% (dry wt).

Crystals of **3** were first found by Johnson [2] in the residue left after the commercial extraction of caffeine from "several million pounds of tea". Uric acid (**4**) or its derivatives are expectable intermediates in the catabolism of methylated xanthines. Urinary breakdown products of caffeine in man are mainly 1-methyluric acid (**5**) and 1-methylxanthine (**6**) [3], whereas recent studies [4,5] with labelled caffeine in the rat indicate that it can be oxidized to the corresponding methylated uric acid. Kalberer [6] synthesized four variously ^{14}C-labelled caffeines and studied their degradation in the leaves of *Coffea arabica*. In addition to allantoin, 3- and/or 7-methylxanthine (**7**,**8**) were identified as breakdown products, whereas no radioactivity was detected in the various mono- and dimethyluric acids. The two methylated uric acids reported here could be the first products of caffeine catabolism, a hypothesis which we are now testing.



Scheme 1.

The proper identification of **2** was relatively laborious. The analysis and spectral data indicated the molecular formula $C_8H_{10}N_4O_3$. The presence of amide groups and of three N or O bonded methyl groups could easily be demonstrated by NMR and IR spectra. The UV spectrum clearly indicated that **2** was not one of the possible trimethyluric acids, or one of the three *N*,*N*-dimethyl-8-methoxy-3,7-dihydro-1H-purine-2,6-diones (see Table 1). In order to complete the identification, all the other possible structures had to be synthesized. In Scheme 1 the synthesis of **2** and **3** is described.

Table 1. UV data (MeOH) of purine derivatives

Compound	λ_{max} (nm)	ϵ	λ_{min} (nm)	ϵ
8-Methoxy-1,9-dimethyl-3,7-dihydro-1H-purine-2,6-dione	274 211·5	12680 12960	245	2904
8-Methoxy-3,7-dimethyl-3,7-dihydro-1H-purine-2,6-dione	277 214	13220 13940	247	3714
8-Methoxy-1,3,7-trimethyl-3,7-dihydro-1H-purine-2,6-dione (**13**)	276·5 213	12960 14050	247	2777
1,3,7-Trimethyl-7,9-dihydro-1H-purine-2,6,8(3H)-trione	290 230* 209·5	11500 6950 9773	259	3091
1,3,9-Trimethyl-7,9-dihydro-1H-purine-2,6,8(3H)-trione (**11**)	291 233 210	11570 6794 10720	259 224·5	2512 6063
1,7,9-Trimethyl-7,9-dihydro-1H-purine-2,6,8(3H)-trione	287 236 210	11040 7030 9630	258 222	3470 5270
3,7,9-Trimethyl-7,9-dihydro-1H-purine-2,6,8(3H)-trione	293 234 209	10980 7650 10010	261 221	2346 5003
1,3,7,9-Tetramethyl-7,9-dihydro-1H-purine-2,6,8(3H)-trione (**3**)	292·5 231 211	9965 6976 11860	262 226	2740 6078
2-Methoxy-1,9-dimethyl-7,9-dihydro-1H-purine-6,8-dione (**2**)	282 245 212·5	8155 7787 13520	261 225	4533 3574
2-Methoxy-1,7,9-trimethyl-7,9-dihydro-1H-purine-6,8-dione (**12**)	284·5 247·5 213	8145 8428 17080	265 226	4807 3596

* Shoulder.

Table 2. Methyl absorption in NMR spectra (60 or 100 MHz) of purine derivatives*

Compound	Solvent	Methyl absorption
1,3,7-Trimethyl-3,7-dihydro-1H-purine-2,6-dione (caffeine) (**1**)	TFA†	4·30 3·74 3·53
8-Methoxy-1,9-dimethyl-3,7-dihydro-1H-purine-2,6-dione	TFA	4·39 3·86 3·51
8-Methoxy-3,7-dimethyl-3,7-dihydro-1H-purine-2,6-dione [15]	TFA	4·11 3·64 3·46
8-Methoxy-1,3,7-trimethyl-3,7-dihydro-1H-purine-2,6-dione (**13**)	TFA	4·47 3·89 3·74 3·56
1,9-Dimethyl-7,9-dihydro-1H-purine-2,6,8(3H)-trione (**9**)	TFA	3·58 3·56
1,3,7-Trimethyl-7,9-dihydro-1H-purine-2,6,8(3H)-trione [16]	TFA	3·71 3·65 3·55
1,3,9-Trimethyl-7,9-dihydro-1H-purine-2,6,8(3H)-trione (**11**)	TFA	3·98 3·85 3·59
1,7,9-Trimethyl-7,9-dihydro-1H-purine-2,6,8(3H)-trione	TFA	3·75 3·60 3·56
3,7,9-Trimethyl-7,9-dihydro-1H-purine-2,6,8(3H)-trione	TFA	3·93 3·88 3·75
1,3,7,9-Tetramethyl-7,9-dihydro-1H-purine-2,6,8(3H)-trione (**3**)	TFA	3·89 3·79 3·70 3·52
2-Methoxy-1,9-dimethyl-7,9-dihydro-1H-purine-6,8-dione (**2**)	$CDCl_3$	4·08 3·50 3·37
2-Methoxy-1,7,9-trimethyl-7,9-dihydro-1H-purine-2,6,8(3H)-trione (**12**)	$CDCl_3$	4·04 3·58 3·43 3·34

* Chemical shifts in ppm relative to internal TMS.
† TFA = trifluoroacetic acid.

In Tables 1 and 2, UV and NMR data are presented.

EXPERIMENTAL

General. Mps are corrected. IR were measured in KBr discs and in $CHCl_3$ soln, UV in MeOH (Table 1); for NMR spectra, see Table 2. The MS were recorded by direct inlet method at 70 eV ionization potential. For TLC Si gel HF_{254} "nach Stahl (Merck)" was used. PLC was carried out using Si gel 60 PF_{254} (Merck) plates and $CHCl_3$ and $CHCl_3$–MeOH mixtures. The spots were detected in UV light.

Plant material. Fruits and leaves of the different *Coffea* species were obtained from the Agricultural Experimental Station at Lyamungu, Tanzania, from the field station of the "Institut Français du café, du cacao et d'autres plantes stimulantes" at Kianjavato, Madagascar, and from the Botanical Gardens, Bogor, Java.

Isolation. Plant material was boiled for 20 min in acidified H_2O (100 ml H_2O + 25 ml 0·05 N H_2SO_4/g dry wt). After cooling 13 g MgO were added and the slurry was filtered. The filtrate was extracted with $CHCl_3$ (20 ml × 5). After concentrating, the $CHCl_3$ ext. was chromatographed on Merck TLC sheets 60 F_{254} with Me_2CO–$CHCl_3$–*n*-BuOH–NH_4OH (3:3:4:1). The faster running **3** was crystallized from EtOH and the slower running **2** was crystallized from EtOH + few drops of H_2O. For analyses, the substances were sublimed at 120–140°/10^{-3} Torr.

Characterization. **2**, colourless needles, mp 269–273°, melts and resolidifies due to thermal rearrangement into 1,3,9-trimethyl-7,9-dihydro-1H-purine-2,6,8(3H)-trione (**11**) [7]. **2** was sublimed at 120–140°/10^{-3} Torr without thermal rearrangement. UV and NMR, see Tables 1 and 2. IR: ν_{max}^{KBr} 3142, 2965, 1693, 1618, 1560 and 1529 cm^{-1}. MS: M^+ 210 (100%), $C_8H_{10}N_4O_3$ (high resolution) *m/e* (relative abundance in %) 195 (5), 181 (10), 168 (5), 167 (13), 166 (7), 153 (5), 152 (8), 139 (5), 125 (6), 124 (11), 123 (5), 110 (5), 105 (5), 97 (5), 96 (8), 83 (43), 72 (18), 70 (12), 69 (10), 68 (17), 67 (18), 58 (12), 56 (17), 54 (13), 53 (20), 42 (20). (**3**), Colourless needles (EtOH) mp 229–230°. UV and NMR, see Tables 1 and 2. IR: $\nu_{max}^{CHCl_3}$ 1735, 1697, 1670 and 1547 cm^{-1}. MS: M^+ 224 (100%) *m/e* (%) 209 (5), 195 (4), 181 (4), 167 (20), 166 (10), 140 (6), 139 (20), 112 (8), 111 (6), 98 (4), 83 (22), 82 (76), 81 (8), 70 (29), 69 (16), 68 (5), 67 (61), 66 (7), 56 (14), 42 (27).

Thermal rearrangement and methylation of **2**. **2** (10 mg) Was heated at 310°/10^{-3} Torr for 10 min in a sealed Pyrex tube, after which the residue was purified by PC (SiO_2; $CHCl_3$, MeOH 10%) to give 1,3,9-trimethyl-7,9-dihydro-1H-purine-2,6,8(3H)-trione (**11**) (6 mg), identified with the authentic specimen in MS, TLC and IR.

Compound **11** (10 mg) in NaOH (4 ml; 1 N) and Me_2SO_4 (40 mg) were stirred at 20° for 45 min. The crude methylated product, 2-methoxy-1,7,9-trimethyl-7,9-dihydro-1H-purine-6,8-dione (**12**) was subjected to PC (SiO_2; $CHCl_3$, MeOH 10%). The main fraction gave **12** as a colourless solid which was crystallized from MeOH (10 mg), mp 207·3–208·4° (lit. mp 186–197° [8–10]) thermally rearranging to 1,3,7,9-tetramethyl-7,9-dihydro-1H-purine-2,6,8(3H)-trione (**3**). UV and NMR: see Tables 1 and 2. IR: ν_{max}^{KBr} 1721, 1690, 1619, 1560 and 1530 cm^{-1}. MS: M^+ 224 (100%), *m/e* (%) 209 (12), 195 (11), 181 (32), 168 (9), 167 (7), 166 (11), 152 (6), 139 (9), 124 (10), 112 (7), 96 (11), 83 (65), 82 (20), 72 (15), 70 (10), 69 (7), 68 (6), 67 (26), 56 (16), 42 (15). **12** (11 mg) was heated at 210°/10^{-3} Torr in a sealed Pyrex tube for 10 min, cooled and purified by PC (SiO_2; $CHCl_3$, MeOH 10%) to give the starting material (**12**) (3 mg) R_f 0·61 and 1,3,7,9-tetramethyl-7,9-dihydro-1H-purine-2,6,8(3H)-trione (**3**) (2 mg) R_f 0·44; mp 229–230°. TLC in different solvents and the IR in $CHCl_3$ were identical with the naturally obtained **3** and with the synthetic sample obtained from 8-methoxycaffeine [11,12].

Synthesis of the purine derivatives **2**, **11** *and* **12**. 1,9-Dimethyl-7,9-dihydro-1H-purine-2,6,8(3H)-trione (1,9-dimethyl-uric acid) [13] (**9**) (150 mg) was refluxed with Ac_2O (75 ml) for 5 hr and the soln was concentrated to 5 ml; on addition of Et_2O colourless crystals separated out, which on standing overnight changed into a different crystalline form. This was filtered, washed (Et_2O) and dried in high vacuum giving 1,9-dimethyl-7-acetyl-7,9-dihydro-1H-purine-2,6,8(3H)-trione (**10**) (175 mg) mp 282·4–284·7° (lit. mp 282°) [7]. IR: ν_{max}^{KBr} 3440, 1754, 1723, 1713, 1670, 1648 and 1590 cm^{-1}. MS: M^+ 238 (11), *m/e* (%). 196 (73), 167 (6), 166 (9), 153 (30), 139 (36), 111 (14), 110 (11), 97 (5), 84 (6), 83 (32), 82 (5), 70 (9), 69 (8), 68 (17), 67 (21), 58 (31), 57 (10), 56 (20), 55 (12), 54 (24), 53 (16), 43 (100). **10** (130 mg) Was ground finely and treated with dry ethereal CH_2N_2 [14] (20 ml) and stirred for 3 1/2 hr. The soln after evaporation gave a colourless residue which showed three major spots on TLC and were separated by PC (SiO_2; $CHCl_3$, MeOH 5%). The zone corresponding to R_f 0·21 yielded 1,3,9-trimethyl-7,9-dihydro-1H-purine-2,6,8(3H)-trione (**11**) (30 mg) (26%) which was identical in TLC, IR (KBr), UV (MeOH) and MS with the authentic sample [15]. UV and NMR, see Tables 1 and 2. IR: ν_{max}^{KBr} 3430, 1692, 1678 and 1559 cm^{-1}. MS: M^+ 210 (100%), *m/e* (%), 181 (4), 153 (17), 152 (15), 126 (6), 125 (13), 124 (22), 105 (3), 98 (5), 97 (3), 83 (8), 70 (11), 69 (9), 68 (44), 67 (11), 58 (5), 57 (4), 56 (7), 54 (4), 53 (17), 42 (13).

The zone of R_f 0·35 yielded 2-methoxy-1,9-dimethyl-7,9-dihydro-1H-purine-6,8-dione (**2**) (40 mg) (35%). After recrystallization from H_2O its mp, spectral data and TLC (SiO_2; $CHCl_3$–10% MeOH; $CHCl_3$–Me_2CO–MeOH, 1:1:1 and C_6H_6–EtOAc–MeOH, 2:2:1) showed identity with the natural compound **2**. (Found: C, 46·05; H, 4·75; N, 26·72. Calc. for $C_8H_{10}N_4O_3$: 45·72; H, 4·76; N, 26·67%.) Thermal reaction and methylation of synthetic **2** carried out under the same conditions as for natural **2** gave 1,3,9-trimethyl-7,9-dihydro-1H-purine-2,6,8(3H)-trione (**11**) and 2-methoxy-1,7,9-trimethyl-7,9-dihydro-1H-purine-6,8-dione (**12**), respectively, identified by mp, TLC and IR spectra. The third main zone, R_f 0·61, afforded 2-methoxy-1,7,9-trimethyl-7,9-dihydro-1H-purine-6,8-dione (**12**) (7·5 mg) (6%), identical with the methylated product of the natural compound **2**, in mp (207–208°), TLC, IR and also in the thermal rearrangement reaction, giving 1,3,7,9-tetramethyl-7,9-dihydro-1H-purine-2,6,8(3H)-trione (**3**).

Purine derivative **12** was also prepared according to Biltz and Max [8] and purified by PC. After crystallization from MeOH its mp was 206·5–207·5°. The low mp for the same compound in the above literature may be due to impurities. The two **12** preparations were identical.

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